# JOURNAL OF THE SOUTH AFRICAN CHEMICAL INSTITUTE

NEW
SERIES
VOLUME VII
NUMBER ... 2

September
1954
September

NUWE
SERIE
DEEL VII
NOMMER .. 2

*President* — S. S. Israelstam, M.Sc., Ph.D., F.R.I.C. — *President*
*Vice-President*-F. W. Hayes, F.R.I.C., A.M.I.Chem.E.-*Vise-President*
*Hon. Secretary*—C. F. Lindemann, B.Sc.—*Ere-sekretaris*

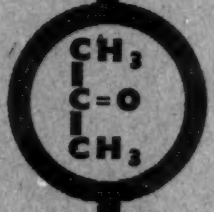
*Publications Committee/Publikasie-komitee:*
S. S. Israelstam, M.Sc., Ph.D., F.R.I.C. (*Chairman/Voorsitter*);
S. Goodman, B.Sc. (Eng.);
F. Hawke, M.Sc. (Eng.), Ph.D., F.R.I.C., A.M.I.Chem.E.;
A. W. Lategan, M.Sc., Ph.D.;
J. A. McLachlan, D.Sc. (Eng.)., M.I.Chem.E.;
K. A. Murray, M.Sc., Ph.D., A.R.I.C.;
M. Mrost, M.Sc.;
G. W. Perold, D.Sc., Dr. sc.techn.;
A. J. Petrick, M.Sc., Dipl. Ing., Dr. Ing.;
*the office Bearers/die Ampsdraers;*
*and the Editor of the Journal/en die Redakteur van die Joernaal,*
H. A. E. Mackenzie, M.Sc., Ph.D., D.I.C., A.R.I.C.

Published by the Institute, Kelvin House, Johannesburg
Uitgegee deur die Instituut, Kelvinhuis, Johannesburg
*Price* / *Prys* } 7/6

# JOERNAAL VAN DIE SUID-AFRIKAANSE CHEMIESE INSTITUUT

# Acetone

B.S.S. 509/1956




## SPECIFICATIONS

SPECIFIC GRAVITY 20°/20° : 0.791 — 0.793
DISTILLATION RANGE: Not less than 95% between 55.5—56.5°C.
ACIDITY (As Acetic Acid): Max. 0.002%
WATER CONTENT: No turbidity with 10 vols $CS_2$.
PERMANGANATE TEST: At least thirty minutes.

## USES

As a solvent for Nitro-cellulose in the manufacture of lacquers. As a paint remover. As a solvent for Acetylene in Welding Industry. For Artificial Leather Artificial Silk, Explosives, Synthetic Resins and Thinners.

**N.C.P.**

Head Office
GERMISTON, TRANSVAAL

## NATIONAL CHEMICAL PRODUCTS LIMITED

**Industrial Fermentation Specialists and producers of organic solvents**

Subsidiary Company: UMGENI DISTILLERIES LTD., SEA COW LAKE ROAD, BOX 2204, DURBAN
Cape Town Office: National Chemical Products Ltd., 832 Grand Parade Centre, Adderley Street, Cape Town
Port Elizabeth Office: National Chemical Products Ltd., Martian House, Strand St., Port Elizabeth.
Agents: Poly Resin Products Ltd., East London. Universal Agencies, Bloemfontein. Basil Frost Limited, Salisbury
Leslie & Anderson (East Africa) Limited, Mombasa.

*Die Redakteur en die Suid-Afrikaanse Chemiese Instituut aanvaar geen verantwoordelikheid vir verklarings wat gemaak word of menings wat uitgespreek word deur bydraers nie.*

*The Editor and the South African Chemical Institute assume no responsibility for statements made and opinions expressed by contributors.*

---

## CONTENTS — INHOUD


*Page/Bladsy*

THE CALIBRATION OF A STORMER VISCOSIMETER MODIFIED FOR TESTING SUSPENSIONS
by P. J. van der Walt and A. M. Fourie .. .. .. .. 61

THE FLAME PHOTOMETRIC DETERMINATION OF CALCIUM IN PLANT AND BIOLOGICAL MATERIALS
by A. Strasheim and J. P. Nell .. .. .. .. .. 79

THE COMPOSITION OF THE BASES FROM AN ISCOR COAL TAR
by F. W. Hougen and (Mrs.) M. A. Raal .. .. .. .. 90

A RELATIONSHIP BETWEEN SUTHERLAND'S CONSTANT AND THE MAXIMUM ENERGY OF ATTRACTION BETWEEN GAS MOLECULES
by C. J. G. Raw .. .. .. .. .. .. .. 96

CHEMISTRY OF NARAS SEED (*ACANTHOSICYOS HORRIDA*, HOOK)
Part I. A Physico-Chemical Study of the Proteins from Naras Seed.
by F. J. Joubert and D. C. Cooper .. .. .. .. 99

ELECTROPHORETIC PROPERTIES AND MOLECULAR WEIGHTS OF ACACIA PLANT GUMS
by F. J. Joubert .. .. .. .. .. .. .. 107

UPTAKE AND DEGRADATION OF LABELLED SYSTEMIC INSECTICIDES
Part II. Treatment of Tobacco and Potatoes with *Systox*.
by L. H. Stein and A. J. Smith .. .. .. .. .. 114
Part III. Estimation of the More Toxic Degradation Products of *Systox*
by L. H. Stein .. .. .. .. .. .. .. 120

FERTILIZER STUDIES WITH TOBACCO PLANTS USING RADIO-PHOSPHORUS-LABELLED SUPERPHOSPHATE
Part IV.
by L. H. Stein and H. L. Pearse .. .. .. .. .. 125

BITTER PRINCIPLES OF THE CUCURBITACEAE
Part II. Paper Chromatography of Bitter Principles and Some Applications in Horticultural Research.
by P. R. Enslin, T. G. Joubert and S. Rehm .. .. .. 131



## EDITORIAL ADVISORY BOARD
## REDAKSIONELE RAADGEWENDE BESTUUR

The Editor of the Journal/Die Redakteur van die Joernaal,
*Chairman/Voorsitter.*
R. du Toit, B.Sc., B.Sc.(Eng.)
G. W. Perold, D.Sc., Dr. sc.,techn.
and/en
The Chairman of the Publications Committee/Die Voorsitter van die Publikasie-komitee (*ex officio*).

# THE NATAL CHEMICAL SYNDICATE LTD.

Manufacturers of

Industrial Alcohol, Rectified Spirit and Methylated Spirit.

—oOo—

**SECRETARIES and SELLING AGENTS:**

HUNT, LEUCHARS and HEPBURN, LIMITED.

Phone 27511. P.O. Box 943.

DURBAN.



FOR ALL YOUR LABORATORY REQUIREMENTS:—
VIR AL U LABORATORIUMBENODIGHEDE:—

## HICKMAN & KLEBER (EDMS.) BPK. (PTY.) LTD.

"The Chemical Supply House"
„Die Chemiese Voorsieners"

| *APPARATUS* *APPARAAT* | *GLASSWARE* *GLAS* | *CHEMICALS* *CHEMIKALIEË* |
|---|---|---|
| Phones 25380 Telefone 61797 | 271 UMBILO ROAD UMBILOWEG 271 DURBAN | P.O. Box 2805 Posbus |

*T.A. Techserv*

# THE CALIBRATION OF A STORMER VISCOSIMETER MODIFIED FOR TESTING SUSPENSIONS

*by*

**P. J. van der WALT and A. M. FOURIE**

## OPSOMMING

Dit is moontlik gevind om suspensies, wat gewoonlik onstabiel is, in die bakkie van 'n Stormerviskosimeter homogeen versprei te hou met 'n roerder wat op sy bodem ingebou word. Hierin word ook aangetoon dat dit onder hierdie besondere toetsomstandighede moontlik is om 'n kalibreringskromme te bepaal wat die verhouding tussen 'n weerstandskoëffisiënt Q en die Reynolds-getal **Re** aandui, mits die standaardvergelykings wat vir die verkryging van die waardes Q en **Re** gebruik word, doelmatig gewysig is.

Deur gebruik te maak van hierdie metode blyk dit wel moontlik om die skynbare viskositeit van 'n suspensie met 'n redelike mate van akkuraatheid te bepaal.

## SUMMARY

It has been found possible to maintain a normally unstable suspension uniformly dispersed in the test cup of a Stormer viscosimeter by introducing a propeller at its base. It is shown that a calibration curve relating a coefficient of resistance, Q, to the Reynolds' Number, **Re**, may be drawn under these test conditions, provided that the standard equations used for deriving appropriate values of Q and **Re** are suitably modified. Making use of this procedure it appears possible to determine the apparent viscosity of a suspension with reasonable accuracy.

## INTRODUCTION

In a previous paper[1] on the calibration of the Stormer viscosimeter under test conditions suitable for true liquids, the authors were able to show that the useful range of the instrument can be extended to include flow conditions in the near turbulent region, by making use of a curve relating a coefficient of resistance, Q, to the Reynolds' Number, **Re**.

Values of Q and **Re**, required to establish the calibration curve, are derived by inserting relevant test data, obtained for liquids of known viscosity and density, in the equations:—

$$\mathbf{Re} = B\rho/\eta t \qquad (1)$$

$$\text{and } Q = Ft^2/B^2\rho, \qquad (2)$$

where $\rho$ = density of test fluid,

$\eta$ = viscosity of the fluid (poise),

t = time required for the rotor to make a fixed number of revolutions (normally 100) in the test fluid,

F = effective driving weight applied to the viscosimeter (actual driving weight minus frictional resistance)*

and B = a constant for a particular rotor, test cup, *etc.*

When the calibration curve is established the viscosity of a liquid is readily determined provided its density is known. It is merely necessary to determine the speed of the rotor when immersed in the liquid and a known driving force has been applied. The value of Q may then be calculated from equation (2) and the corresponding value of **Re** may be read off the calibration curve. The viscosity of the liquid is then calculated, applying equation (1).

Extending this study to the determination of the "apparent viscosity" of unstable suspensions of finely ground solids in water, it soon became clear that a substantial modification of the equipment, the testing and the calibration procedures would be necessary, before this instrument could be used with any confidence.

* Strictly, F is only proportional to the effective driving weight.

A practical approach to these problems was adopted and proof was obtained that this viscosimeter may be converted into a useful tool for determining the apparent viscosity of suspensions.

## MODIFICATIONS TO THE EQUIPMENT AND TESTING PROCEDURE

### Problems arising from the Instability of Suspensions

Determinations of the apparent viscosity would only have some meaning provided that the suspension remained reasonably stable during the test period. The degree of stability was checked by conducting tests in the standard viscosimeter during which samples of the suspension were abstracted from the cup at regular intervals during the test period. These samples represented the portion of the suspension surrounding the rotor. The average density of the samples was determined by weighing each sample and subsequently the dry solids it contained.

The following typical results were obtained for a suspension of silica flour in water made up to a specific gravity of 1·5:

| Time sampled after commencement of test (sec.) | Average specific gravity |
|---|---|
| 0 | 1·503 |
| 20 | 1·475 |
| 40 | 1·429 |
| 60 | 1·394 |

It was concluded that such a variation would render determinations meaningless and that it would therefore be necessary to agitate the suspension in some way during tests.

The idea of using the rotor (possibly in some modified form) for this purpose was abandoned because there was already reason to believe that the apparent viscosity of a suspension is not constant but varies with the speed of the rotor (or the rate of shear) and this could only be confirmed by having rotor speed in hand as a variable.

After considering other alternatives the method of agitation indicated in *Fig.* 1 was finally adopted. The modification amounts, essentially, to providing a two-bladed propeller at the base of the test cup driven (at variable speed) from an external source and the attachment of four baffles to the inner surface of the cup to prevent the creation of a vortex.

Notwithstanding these baffles, the propeller apparently sets up a rotational motion in the liquid in the cup which is imparted to the rotor. The propeller has therefore been made to rotate in a direction opposite to the normal direction of rotation of the rotor. A certain driving weight is then required on the pan to keep the rotor stationary and it is possible to cause it to rotate at low speed in its normal direction by the addition of small extra driving weights.

The stability of suspensions in this modified apparatus was checked in the same way as before, and it was found that a homogeneous suspension could readily be maintained. In some instances low propeller speeds proved adequate while in others speeds of the order of 500 r.p.m. were required.

The following is a typical example of the results obtained. Using a suspension of silica flour in water having a specific gravity of 1·5 and a propeller speed of 400 r.p.m. the average specific gravity of samples abstracted from the cup over a test period of 30 minutes varied from 1·502 to 1·505.

**Discrepancies due to degree of Immersion of the Rotor**

In previous work the cylindrical rotor was not entirely immersed in the liquid. The results of duplicate tests did not always agree well enough and larger discrepancies were observed when the propeller was introduced to agitate the liquid since this frequently disturbed the surface of the liquid. Consequently it was decided to immerse the cylinder completely. In this way only the thin stem (having a much smaller surface area per unit length) is involved in variations of liquid level (see *Fig.* 1). This modification considerably improved the reproducibility of the results.

**Discrepancies due to temperature effects**

It was observed that large variations in ambient temperature affected the frictional resistance of the instrument. It was also found difficult to control the temperature of the test liquid within narrow limits by means of a water jacket surrounding the cup alone. The instrument was therefore placed in a constant temperature room (23°C. $\pm$2°C.) and, in addition the temperature of the water in the jacket was maintained constant. In this way the temperature of the test liquid could be maintained at 25°C. $\pm$0·2°C. and more consistent results were obtained.

**The Frictional Resistance of the Instrument**

In the course of this study, relatively small driving weights were used from time to time; it was therefore necessary to establish the frictional resistance of the instrument with sufficient accuracy.

Preliminary tests indicated that the average value of the frictional resistance of this particular instrument while in operation is of the order of 0·9 gram acting at the radius of the drum. This value was deduced from the driving weights applied during the tests. The indications are, however, that the frictional resistance varies with factors such as the magnitude of the driving weight and possibly the buoyancy of the rotor in the test liquid. These aspects are receiving attention.

## CALIBRATION OF THE MODIFIED INSTRUMENT

In order to calibrate the instrument tests were conducted using true liquids of known viscosity and density.

Three series of tests were run using a cylindrical rotor. In the first series the propeller was stationary while in the others the propeller speeds were 400 r.p.m. and 520 r.p.m. respectively. The results of these tests together with other relevant data are presented in Tables I, II and III.

As long as the propeller was stationary equations (1) and (2) could be used successfully to determine the calibration curve of the instrument along the lines mentioned briefly in the introduction to this paper. (See *Fig.* 2 which was plotted from the data in Table I).

When, however, these equations were used to calculate values of Q and **Re** from data obtained during the second and third series of tests it was impossible to obtain a simple calibration curve covering all the liquids with accuracy. Referring to *Fig.* 3, it appeared as if a different relationship between Q and **Re** existed for every liquid.

It was concluded that this result might be due to shortcomings in the equations. Since the liquid in the cup probably had a positive rotational motion (imparted by the propeller) opposite to that of the rotor, the idea was suggested that the "relative velocity" between rotor and fluid should be used to evaluate results instead of the rotor speed given by the revolution counter. Whenever a counter-rotational motion exists in the liquid the relative velocity will be higher than the speed of the rotor by some factor, $x$.

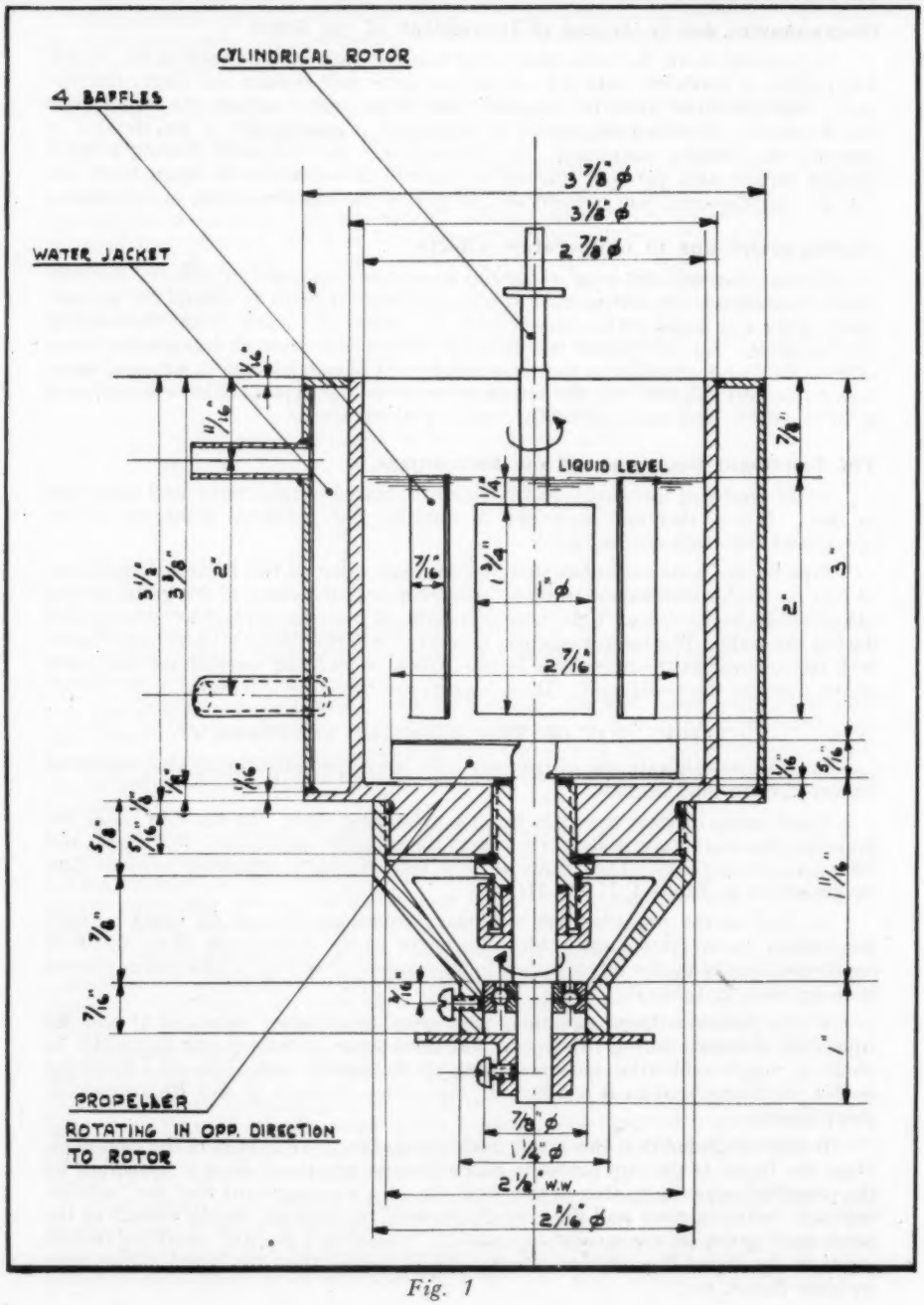

CYLINDRICAL ROTOR
4 BAFFLES
WATER JACKET
3 7/8" ϕ
3 1/8" ϕ
2 7/8" ϕ
LIQUID LEVEL
1/4"
7/16"
1 3/4"
1" ϕ
2 7/16"
2"
3"
7/8"
11/16"
3 1/8"
3 3/8"
5/16"
5/8"
7/16"
1 11/16"
1"
PROPELLER
ROTATING IN OPP. DIRECTION
TO ROTOR
7/8" ϕ
1 1/4" ϕ
2 1/8" W.W.
2 5/16" ϕ

*Fig. 1*

TABLE I

RESULTS OBTAINED WITH CYLINDRICAL ROTOR (PROPELLER STATIONARY)

| Liquid | Viscosity* at 25°C. (cS.) | Density ($\rho$) at 25°C. (g. cm$^{-3}$) | Viscosity at 25°C. (cP.) | Effective Driving Weight (F) (g.) | Time for 100 Revs. (t) (sec.) | Re/B | $\rho/t^2$ | QB$^2$ | Viscosity derived from curve (cS.) | % Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Solution of Glycerine in water .. .. | 45·38 | 1·212 | 55·00 | 35·1 | 17·6 | 0·1252 | 0·0039129 | 8,970 | 45·46 | 0·2 |
| | | | | 30·1 | 19·8 | 0·1113 | 0·0030922 | 9,730 | 45·30 | 0·2 |
| | | | | 25·1 | 22·75 | 0·0969 | 0·0023422 | 10,720 | 45·41 | 0·1 |
| | | | | 21·1 | 25·85 | 0·0852 | 0·0018133 | 11,640 | 45·51 | 0·3 |
| | | | | 17·1 | 30·3 | 0·0727 | 0·0013199 | 12,960 | 45·21 | 0·4 |
| | | | | 14·1 | 35·1 | 0·0628 | 0·0009838 | 14,330 | 45·22 | 0·4 |
| | | | | 11·1 | 42·3 | 0·0521 | 0·0006773 | 16,390 | 45·46 | 0·2 |
| Solution of Glycerine in water .. .. | 44·05 | 1·212 | 53·39 | 35·1 | 17·25 | 0·1316 | 0·0040744 | 8,610 | 43·73 | 0·7 |
| | | | | 30·1 | 19·35 | 0·1173 | 0·0032370 | 9,300 | 43·43 | 1·4 |
| | | | | 26·1 | 21·55 | 0·1053 | 0·0026094 | 10,010 | 43·36 | 1·6 |
| | | | | 22·1 | 24·4 | 0·0930 | 0·0020354 | 10,860 | 43·14 | 2·1 |
| | | | | 19·1 | 27·2 | 0·0835 | 0·0016378 | 11,660 | 43·25 | 1·8 |
| | | | | 16·1 | 31·0 | 0·0732 | 0·0012613 | 12,760 | 43·30 | 1·7 |
| | | | | 13·1 | 36·4 | 0·0624 | 0·0009146 | 14,320 | 43·26 | 1·8 |
| | | | | 10·1 | 44·5 | 0·0510 | 0·0006119 | 16,510 | 44·06 | 0·0 |
| Solution of Glycerine in water .. .. | 37·88 | 1·207 | 45·72 | 30·1 | 17·9 | 0·1475 | 0·0037677 | 7,990 | 37·62 | 0·7 |
| | | | | 26·1 | 20·0 | 0·1320 | 0·0030175 | 8,650 | 37·88 | 0·0 |
| | | | | 22·1 | 22·7 | 0·1163 | 0·0023421 | 9,440 | 37·81 | 0·2 |
| | | | | 18·1 | 26·4 | 0·1000 | 0·0017319 | 10,450 | 37·69 | 0·5 |
| | | | | 14·1 | 32·0 | 0·0825 | 0·0011788 | 11,960 | 38·11 | 0·6 |
| | | | | 11·1 | 38·5 | 0·0686 | 0·0008140 | 13,640 | 38·47 | 1·6 |
| Solution of Glycerine in water .. .. | 32·73 | 1·203 | 39·37 | 30·1 | 16·7 | 0·1830 | 0·0043135 | 6,980 | 32·72 | 0·0 |
| | | | | 25·1 | 19·0 | 0·1608 | 0·0033322 | 7,530 | 32·59 | 0·4 |
| | | | | 21·1 | 21·75 | 0·1405 | 0·0025422 | 8,300 | 32·67 | 0·2 |
| | | | | 17·1 | 25·45 | 0·1200 | 0·0018571 | 9,210 | 32·55 | 0·6 |
| | | | | 14·1 | 29·5 | 0·1036 | 0·0013825 | 10,200 | 32·60 | 0·4 |
| | | | | 11·1 | 35·3 | 0·0866 | 0·0009655 | 11,500 | 32·64 | 0·3 |
| | | | | 9·1 | 41·25 | 0·0742 | 0·0007104 | 12,810 | 32·75 | 0·1 |

* Determined in an Ubbelohde viscosimeter.

TABLE I (*Continued*)

| Liquid | Viscosity* at 25°C. (cS.) | Density ($\rho$) at 25°C. (g. cm$^{-3}$) | Viscosity at 25°C. (cP.) | Effective Driving Weight (F) (g.) | Time for 100 Revs. (t) (sec.) | Re/B | $\rho/t^2$ | QB$^2$ | Viscosity derived from curve (cS.) | % Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Solution of Glycerine in water .. .. | 25·26 | 1·196 | 30·21 | 25·1 | 16·9 | 0·2342 | 0·0041873 | 5,990 | 25·23 | 0·1 |
| | | | | 21·1 | 19·1 | 0·2073 | 0·0032790 | 6,430 | 25·05 | 0·8 |
| | | | | 17·1 | 22·3 | 0·1775 | 0·0024047 | 7,110 | 25·33 | 0·5 |
| | | | | 14·1 | 25·8 | 0·1534 | 0·0017968 | 7,850 | 25·33 | 0·5 |
| | | | | 11·1 | 31·1 | 0·1273 | 0·0012362 | 8,980 | 25·62 | 1·4 |
| | | | | 9·1 | 36·4 | 0·1087 | 0·0009025 | 10,080 | 25·87 | 2·4 |
| Oil .. .. .. .. | 50·00 | 0·877 | 43·85 | 30·1 | 16·4 | 0·1220 | 0·0032612 | 9,230 | 50·40 | 0·8 |
| | | | | 25·1 | 18·9 | 0·1058 | 0·0024552 | 10,220 | 51·12 | 2·2 |
| | | | | 21·1 | 21·5 | 0·0930 | 0·0018971 | 11,120 | 50·66 | 1·3 |
| | | | | 18·1 | 24·15 | 0·0828 | 0·0015039 | 12,035 | 50·56 | 1·1 |
| | | | | 15·1 | 27·85 | 0·0718 | 0·0011309 | 13,350 | 51·30 | 2·6 |
| | | | | 12·1 | 33·0 | 0·0606 | 0·0008052 | 15,030 | 51·36 | 2·7 |
| | | | | 9·1 | 41·4 | 0·0483 | 0·0005115 | 17,790 | 51·38 | 2·8 |
| Zinc-chloride solution | 15·13 | 1·917 | 29·00 | 20·1 | 22·65 | 0·2918 | 0·0037366 | 5,380 | 15·07 | 0·4 |
| | | | | 15·1 | 27·05 | 0·2443 | 0·0026202 | 5,760 | 14·67 | 3·0 |
| | | | | 11·1 | 33·65 | 0·1964 | 0·0016933 | 6,560 | 14·71 | 2·8 |
| Oil .. .. .. .. | 24·68 | 0·892 | 22·01 | 19·1 | 16·6 | 0·2441 | 0·0032370 | 5,900 | 24·89 | 0·9 |
| | | | | 16·1 | 18·8 | 0·2155 | 0·0025236 | 6,380 | 24·97 | 1·2 |
| | | | | 13·1 | 21·95 | 0·1846 | 0·0018507 | 7,080 | 25·31 | 2·6 |
| | | | | 11·1 | 25·0 | 0·1621 | 0·0014272 | 7,780 | 25·64 | 3·9 |
| | | | | 9·1 | 29·15 | 0·1390 | 0·0010494 | 8,670 | 25·88 | 4·9 |
| Zinc-chloride solution | 9·08 | 1·844 | 16·74 | 25·1 | 16·25 | 0·6778 | 0·0069836 | 3,590 | 9·12 | 0·4 |
| | | | | 21·1 | 18·0 | 0·6119 | 0·0056922 | 3,710 | 9·08 | 0·0 |
| | | | | 17·1 | 20·55 | 0·5359 | 0·0043662 | 3,920 | 9·08 | 0·0 |
| | | | | 14·1 | 23·25 | 0·4737 | 0·0034112 | 4,130 | 9·05 | 0·3 |
| | | | | 11·1 | 27·3 | 0·4034 | 0·0024743 | 4,490 | 9·11 | 0·3 |
| | | | | 9·1 | 31·2 | 0·3530 | 0·0018942 | 4,800 | 9·03 | 0·6 |
| | | | | 7·1 | 37·25 | 0·2957 | 0·0013293 | 5,340 | 9·04 | 0·4 |

* Determined in an Ubbelohde viscosimeter.

TABLE II

**RESULTS OBTAINED WITH CYLINDRICAL ROTOR (PROPELLER ROTATING AT 400 R.P.M.)**

| Liquid | Viscosity* at 25°C. (cS.) | Density ($\rho$) at 25°C (g. cm.$^{-3}$) | Viscosity at 25°C. (cP.) | Effective Driving Weight (F) (g.) | Time for 100 Revs. (t) (sec.) | $\frac{1}{t}$ (sec.$^{-1}$) | x (sec.$^{-1}$) | Viscosity calculated from calibration curve (cS.) | % Error |
|---|---|---|---|---|---|---|---|---|---|
| Solution of Glycerine in water .. .. .. .. | 44·05 | 1·212 | 53·39 | 40·1 | 18·3 | 0·05464 | 0·00879 | 45·69 | 3·7 |
| | | | | 35·1 | 20·9 | 0·04785 | 0·00986 | 45·16 | 2·5 |
| | | | | 30·1 | 23·55 | 0·04246 | 0·00908 | 42·41 | 3·7 |
| | | | | 25·1 | 29·8 | 0·03356 | 0·01150 | 43·61 | 1·0 |
| | | | | 22·1 | 37·3 | 0·02681 | 0·01400 | 47·03 | 6·8 |
| Solution of Glycerine in water .. .. .. .. | 32·73 | 1·203 | 39·37 | 30·1 | 19·5 | 0·05128 | 0·00829 | 32·75 | 0·1 |
| | | | | 26·1 | 22·25 | 0·04495 | 0·00856 | 32·42 | 1·0 |
| | | | | 23·1 | 25·1 | 0·03984 | 0·00900 | 31·57 | 3·5 |
| | | | | 20·1 | 29·1 | 0·03436 | 0·01000 | 30·91 | 5·6 |
| | | | | 17·1 | 36·0 | 0·02778 | 0·01150 | 31·31 | 4·3 |
| | | | | 14·1 | 48·0 | 0·02083 | 0·01320 | 31·96 | 2·4 |
| Zinc-chloride solution .. | 15·13 | 1·917 | 29·00 | 30·1 | 20·4 | 0·04902 | 0·00920 | 15·48 | 2·3 |
| | | | | 25·1 | 23·9 | 0·04184 | 0·00975 | 14·86 | 1·8 |
| | | | | 22·1 | 27·1 | 0·03690 | 0·01046 | 14·46 | 4·4 |
| | | | | 19·1 | 31·7 | 0·03155 | 0·01112 | 14·51 | 4·1 |
| | | | | 16·1 | 39·1 | 0·02558 | 0·01260 | 15·11 | 0·1 |
| Oil .. .. .. .. .. | 24·86 | 0·892 | 22·18 | 19·1 | 19·75 | 0·05063 | 0·00966 | 26·09 | 4·95 |
| | | | | 16·1 | 23·2 | 0·04310 | 0·01035 | 25·32 | 1·85 |
| | | | | 13·1 | 29·05 | 0·03442 | 0·01150 | 25·29 | 1·7 |
| | | | | 11·1 | 35·65 | 0·02805 | 0·01297 | 25·35 | 2·0 |
| | | | | 9·1 | 47·3 | 0·02114 | 0·01391 | 25·76 | 3·6 |

* Deterined in an Ubbelohde viscosimeter.

TABLE III

**RESULTS OBTAINED WITH CYLINDRICAL ROTOR (PROPELLER ROTATING AT 520 R.P.M.)**

| Liquid | Viscosity* at 25°C. (cS.) | Density ($\rho$) at 25°C (g. cm.$^{-3}$) | Viscosity at 25°C. (cP.) | Effective Driving Weight (F) (g.) | Time for 100 Revs. (t) (sec.) | $\frac{1}{t}$ (sec.$^{-1}$) | x (sec.$^{-1}$) | Viscosity calculated from calibration curve (cS.) | % Error |
|---|---|---|---|---|---|---|---|---|---|
| Oil .. .. .. .. .. | 50·00 | 0·877 | 43·85 | 35·1 | 18·0 | 0·05556 | 0·01334 | 50·20 | 0·4 |
| | | | | 30·1 | 20·8 | 0·04808 | 0·01317 | 48·08 | 3·8 |
| | | | | 26·1 | 25·8 | 0·03876 | 0·01584 | 49·77 | 0·5 |
| | | | | 23·1 | 41·4 | 0·03185 | 0·01815 | 50·79 | 1·6 |
| | | | | 20·1 | 41·15 | 0·02430 | 0·02070 | 53·81 | 7·6 |
| Solution of Glycerine in water .. .. .. .. | 45·38 | 1·212 | 55·00 | 45·1 | 18·3 | 0·05464 | 0·01388 | 45·97 | 1·3 |
| | | | | 40·1 | 20·7 | 0·04831 | 0·01431 | 45·12 | 0·6 |
| | | | | 35·1 | 24·4 | 0·04098 | 0·01565 | 44·91 | 1·0 |
| | | | | 30·1 | 30·6 | 0·03268 | 0·01769 | 45·56 | 0·4 |
| | | | | 26·1 | 39·6 | 0·02525 | 0·01990 | 47·18 | 4·0 |
| Solution of Glycerine in water .. .. .. .. | 37·88 | 1·207 | 45·72 | 40·1 | 18·15 | 0·05510 | 0·01350 | 38·32 | 1·2 |
| | | | | 35·1 | 21·0 | 0·04762 | 0·01450 | 38·04 | 0·4 |
| | | | | 30·1 | 25·25 | 0·03960 | 0·01570 | 37·40 | 1·3 |
| | | | | 26·1 | 30·45 | 0·03284 | 0·01716 | 37·06 | 2·2 |
| | | | | 23·1 | 36·8 | 0·02717 | 0·01829 | 37·32 | 1·5 |
| | | | | 20·1 | 47·3 | 0·02114 | 0·01977 | 38·18 | 0·8 |
| Solution of Glycerine in water .. .. .. .. | 25·26 | 1·196 | 30·21 | 30·1 | 19·05 | 0·05249 | 0·01400 | 25·85 | 2·3 |
| | | | | 26·1 | 22·3 | 0·04484 | 0·01558 | 26·15 | 3·5 |
| | | | | 22·1 | 27·3 | 0·03662 | 0·01744 | 25·67 | 1·6 |
| | | | | 19·1 | 33·1 | 0·03021 | 0·01811 | 25·45 | 0·8 |
| | | | | 16·1 | 43·8 | 0·02283 | 0·02000 | 25·93 | 2·7 |
| Zinc-chloride solution .. | 9·08 | 1·844 | 16·74 | 30·1 | 18·45 | 0·05420 | 0·01417 | 9·77 | 7·6 |
| | | | | 25·1 | 21·85 | 0·04577 | 0·01579 | 9·67 | 6·5 |
| | | | | 20·1 | 27·85 | 0·03591 | 0·01800 | 9·71 | 6·9 |

* Determined in an Ubbelohde viscosimeter.

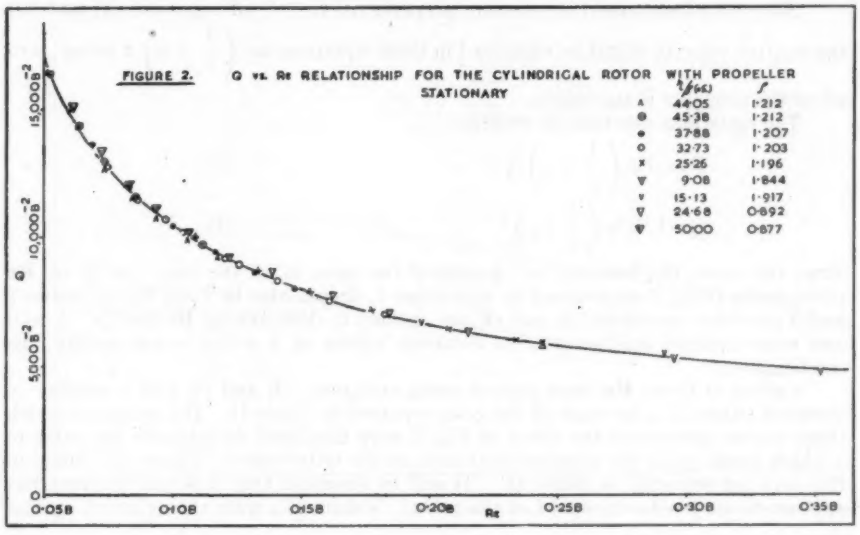


FIGURE 2. Q vs. Re RELATIONSHIP FOR THE CYLINDRICAL ROTOR WITH PROPELLER STATIONARY
Y/(dc) f
44·05 1·212
45·38 1·212
37·88 1·207
32·73 1·203
25·26 1·196
9·08 1·844
15·13 1·917
24·68 0·892
50·00 0·877
Q
15,000 s⁻²
10,000 s⁻²
5,000 s⁻²
0
0·05B
0·10B
0·15B
0·20B
Re
0·25B
0·30B
0·35B

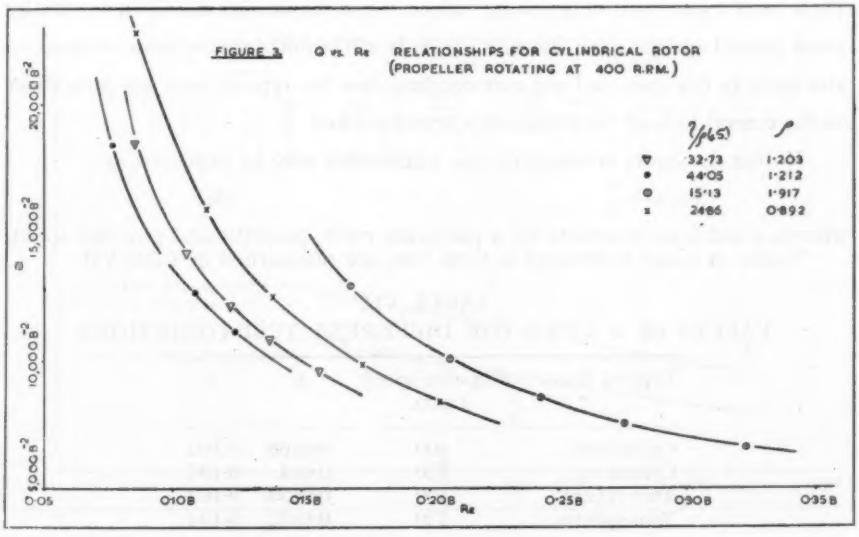


FIGURE 3. Q vs. Re RELATIONSHIPS FOR CYLINDRICAL ROTOR (PROPELLER ROTATING AT 400 R.P.M.)
Y/(dc) f
32·73 1·203
44·05 1·212
15·13 1·917
24·86 0·892
Q
20,000 s⁻²
15,000 s⁻²
10,000 s⁻²
5,000 s⁻²
0·05
0·10B
0·15B
0·20B
Re
0·25B
0·30B
0·35B

Since rotational speed is inversely proportional to "t" (of equations (1) and (2)) the relative velocity might be expressed in these equations by $\left(\frac{1}{t}+x\right)$, $x$ being zero when the propeller is stationary.

The equations can then be written:

$$\mathbf{Re}=B\rho\left(\frac{1}{t}+x\right)/\eta \qquad (3)$$

$$Q=F/B^2\rho\left(\frac{1}{t}+x\right)^2 \qquad (4)$$

Since the rotor, the test cup, *etc.* remained the same in all the tests, the Q vs. **Re** relationship (*Fig.* 2) established in test series 1, should also be valid for the series 2 and 3 provided equations (3) and (4) are applied in determining **Re** and Q. A trial and error method was adopted to establish values of $x$ which would satisfy this condition.

Curves of Q vs. **Re** were plotted using equations (3) and (4) and a number of assumed values of $x$ for each of the tests reported in Table II. The points at which these curves intersected the curve in *Fig.* 2 were then used to estimate the value of $x$ which would place the experimental data on the latter curve. Values of $x$ found in this way are reported in Table II. (It will be observed that $x$ is not constant but appears to vary with the speed of the rotor.) Values of $x$ were then plotted against $\frac{1}{t}$ as shown in *Fig.* 4, which suggests,as a first approximation, a linear relationship between $x$ and $\frac{1}{t}$ within the limits investigated. This procedure was repeated for the third series of tests and again a linear relationship between $x$ and $\frac{1}{t}$ was found.

In order to obtain further data, another series of three sets of experiments was run using a rotor consisting of two spheres mounted offset on a narrow shaft (see *Fig.* 6, Reference [1]). The results of these tests are given in Tables IV, V and VI. These results were used to determine values of $x$ in the manner described and finally $x$ was plotted against $\frac{1}{t}$ as shown in *Fig.* 5. It will be noted that a linear relationship also exists in this case, and one may conclude that the type of rotor has little effect on the general form of the relationship between $x$ and $\frac{1}{t}$.

Within the limits investigated this relationship may be expressed as:

$$x=a-\frac{b}{t} \qquad (5)$$

wherein a and b are constants for a particular rotor, propeller and propeller speed.

Values of a and b obtained in these tests are summarised in Table VII.

TABLE VII

VALUES OF a AND b FOR DIFFERENT TEST CONDITIONS

| Type of Rotor | Propeller speed r.p.m. | a | b |
|---|---|---|---|
| Cylindrical | 400 | 0·0168 | 0·162 |
| Cylindrical | 520 | 0·024 | 0·194 |
| Two spheres | 400 | 0·0133 | 0·162 |
| Two spheres | 520 | 0·0182 | 0·194 |

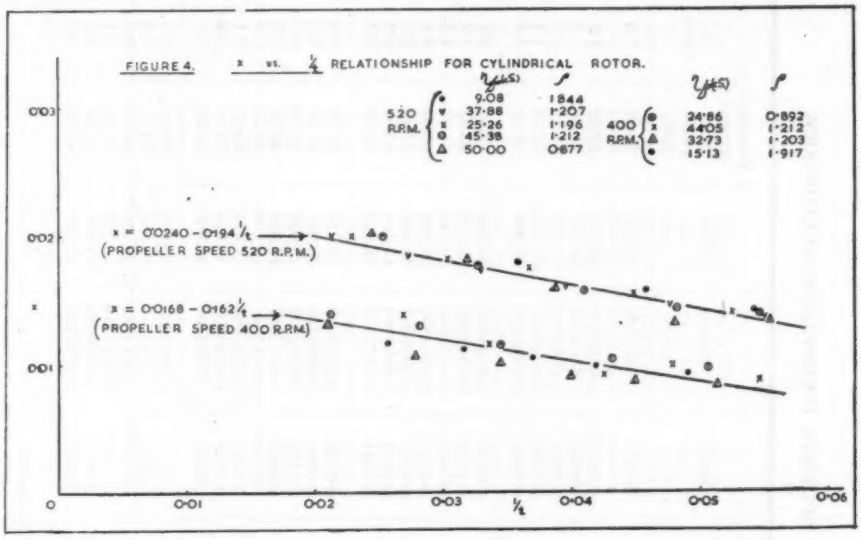


FIGURE 4. x vs. 1/t RELATIONSHIP FOR CYLINDRICAL ROTOR.
η/μ(cS) ρ
520 R.P.M. 9·08 1·844
37·88 1·207
25·26 1·196
45·38 1·212
50·00 0·877
400 R.P.M. 24·86 0·892
44·05 1·212
32·73 1·203
15·13 1·917
x = 0·0240 − 0·194 1/t
(PROPELLER SPEED 520 R.P.M.)
x = 0·0168 − 0·162 1/t
(PROPELLER SPEED 400 R.P.M.)
0·03
0·02
x
0·01
0
0·01
0·02
0·03
1/t
0·04
0·05
0·06

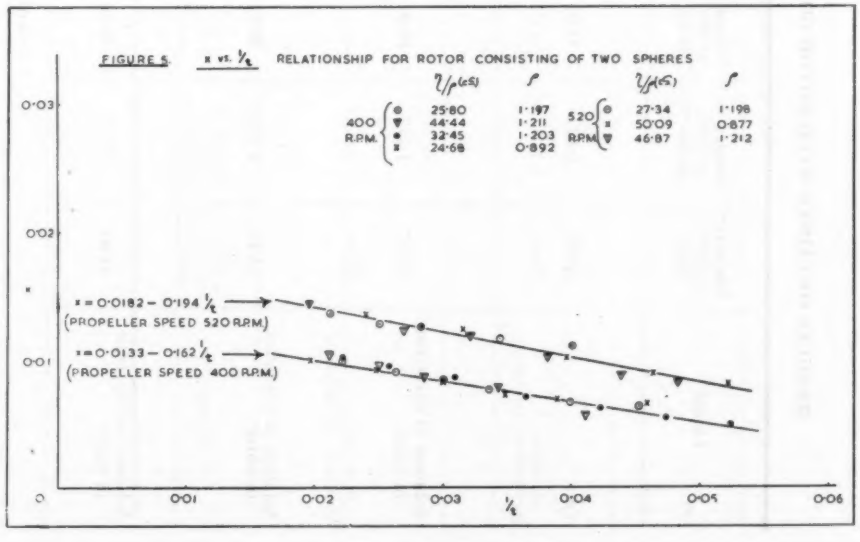


FIGURE 5. x vs. 1/t RELATIONSHIP FOR ROTOR CONSISTING OF TWO SPHERES
η/μ(cS) ρ
400 R.P.M. 25·80 1·197
44·44 1·211
32·45 1·203
24·68 0·892
520 R.P.M. 27·34 1·198
50·09 0·877
46·87 1·212
x = 0·0182 − 0·194 1/t
(PROPELLER SPEED 520 R.P.M.)
x = 0·0133 − 0·162 1/t
(PROPELLER SPEED 400 R.P.M.)
0·03
0·02
x
0·01
0
0·01
0·02
0·03
1/t
0·04
0·05
0·06

TABLE IV

**RESULTS OBTAINED WITH ROTOR CONSISTING OF TWO SPHERES (PROPELLER STATIONARY)**

| Liquid | Viscosity* at 25°C. (cS.) | Density ($\rho$) at 25°C. (g. cm$^{-3}$) | Viscosity at 25°C. (cP.) | Effective Driving Weight (F) (g.) | Time for 100 Revs. (t) (sec.) | Re/B | $\rho/t^2$ | $QB^2$ | Viscosity derived from curve (cS.) | % Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Oil .. .. .. .. | 50·09 | 0·877 | 43·93 | 25 | 47·75 | 0·0418 | 0·0003846 | 65,000 | 49·86 | 0·5 |
| | | | | 35 | 38·5 | 0·0518 | 0·0005914 | 59,180 | 50·14 | 0·1 |
| | | | | 45 | 33·3 | 0·05995 | 0·0007909 | 56,900 | 51·69 | 1·2 |
| | | | | 55 | 29·5 | 0·0677 | 0·0010078 | 54,570 | 51·68 | 1·2 |
| | | | | 75 | 24·5 | 0·0815 | 0·0014613 | 51,320 | 51·15 | 2·1 |
| | | | | 95 | 21·35 | 0·0935 | 0·0019241 | 49,370 | 52·22 | 4·3 |
| | | | | 115 | 19·0 | 0·1051 | 0·0024292 | 47,340 | 51·75 | 3·3 |
| Solution of Glycerine in water .. .. | 46·87 | 1·212 | 56·81 | 35 | 45·8 | 0·0466 | 0·0005775 | 60,610 | 44·92 | 4·2 |
| | | | | 45 | 38·95 | 0·0548 | 0·0007987 | 56,340 | 43·51 | 7·2 |
| | | | | 55 | 34·8 | 0·0613 | 0·0010011 | 54,940 | 44·91 | 4·2 |
| | | | | 75 | 29·05 | 0·0734 | 0·0014359 | 52,230 | 45·89 | 2·1 |
| | | | | 95 | 25·0 | 0·0853 | 0·0019392 | 48,990 | 43·72 | 6·7 |
| | | | | 115 | 22·3 | 0·0957 | 0·0024368 | 47,190 | 43·62 | 6·9 |
| | | | | 135 | 20·3 | 0·1051 | 0·0029409 | 45,900 | 44·06 | 6·0 |
| Solution of Glycerine in water .. .. | 25·49 | 1·197 | 30·51 | 25 | 50·25 | 0·0781 | 0·0004740 | 52,740 | 27·26 | 6·9 |
| | | | | 35 | 40·9 | 0·0959 | 0·0007156 | 48,910 | 26·43 | 3·7 |
| | | | | 45 | 34·95 | 0·1122 | 0·0009797 | 45,930 | 25·50 | 0·0 |
| | | | | 55 | 31·25 | 0·1255 | 0·0012257 | 44,870 | 26·23 | 2·9 |
| | | | | 65 | 28·2 | 0·1391 | 0·0015051 | 43,190 | 25·70 | 0·8 |
| | | | | 85 | 24·05 | 0·1631 | 0·0020695 | 41,070 | 25·51 | 0·1 |
| | | | | 105 | 21·25 | 0·1846 | 0·0026509 | 39,610 | 25·30 | 0·7 |
| | | | | 125 | 19·2 | 0·2043 | 0·0032466 | 38,500 | 25·22 | 1·1 |
| Solution of Glycerine in water .. .. | 44·83 | 1·211 | 54·29 | 45 | 38·7 | 0·0576 | 0·0008085 | 55,650 | 42·02 | 6·3 |
| | | | | 55 | 34·6 | 0·0645 | 0·0010114 | 54,380 | 43·79 | 2·3 |
| | | | | 65 | 31·4 | 0·0710 | 0·0012284 | 52,910 | 44·55 | 0·6 |
| | | | | 85 | 26·45 | 0·0843 | 0·0017312 | 49,100 | 41·64 | 7·1 |
| | | | | 105 | 23·45 | 0·0951 | 0·0022028 | 47,670 | 42·86 | 4·4 |
| | | | | 125 | 21·1 | 0·1057 | 0·0027197 | 45,960 | 42·31 | 5·6 |

* Determined in an Ubbelohde viscosimeter.

TABLE IV (*Continued*)

| Liquid | Viscosity* at 25°C. (cS.) | Density ($\rho$) at 25°C. (g. cm$^{-3}$) | Viscosity at 25°C. (cP.) | Effective Driving Weight (F) (g.) | Time for 100 Revs. (t) (sec.) | Re/B | $\rho/t^2$ | QB$^2$ | Viscosity derived from curve (cS.) | % Error |
|---|---|---|---|---|---|---|---|---|---|---|
| Solution of Glycerine in water .. .. | 32·45 | 1·203 | 39·04 | 30 | 47·3 | 0·0651 | 0·0005376 | 55,800 | 34·54 | 6·4 |
| | | | | 40 | 39·3 | 0·0784 | 0·0007792 | 51,330 | 32·01 | 1·4 |
| | | | | 50 | 34·55 | 0·0892 | 0·0010082 | 49,590 | 32·71 | 0·8 |
| | | | | 60 | 31·15 | 0·0989 | 0·0012396 | 48,400 | 33·58 | 3·5 |
| | | | | 70 | 28·2 | 0·1093 | 0·0015127 | 46,270 | 32·38 | 0·2 |
| | | | | 80 | 25·9 | 0·1190 | 0·0017933 | 44,610 | 31·19 | 3·9 |
| | | | | 100 | 22·7 | 0·1357 | 0·0023343 | 42,840 | 31·20 | 3·9 |
| | | | | 120 | 20·4 | 0·1511 | 0·0028908 | 41,510 | 31·32 | 3·5 |
| | | | | 140 | 18·7 | 0·1648 | 0·0034407 | 40,690 | 31·74 | 2·2 |
| Solution of Glycerine in water .. .. | 25·80 | 1·197 | 30·88 | 35 | 41·5 | 0·0934 | 0·0006952 | 50,350 | 28·35 | 9·9 |
| | | | | 45 | 35·5 | 0·1092 | 0·0009499 | 47,370 | 27·62 | 7·1 |
| | | | | 55 | 31·55 | 0·1229 | 0·0012027 | 45,730 | 27·76 | 7·6 |
| | | | | 65 | 28·45 | 0·1362 | 0·0014789 | 43,950 | 27·04 | 4·8 |
| | | | | 85 | 24·15 | 0·1605 | 0·0020526 | 41,410 | 26·13 | 1·3 |
| | | | | 105 | 21·35 | 0·1815 | 0·0026262 | 39,980 | 25·95 | 0·6 |
| | | | | 125 | 19·3 | 0·2008 | 0·0032131 | 38,900 | 25·97 | 0·7 |
| | | | | 145 | 17·7 | 0·2190 | 0·0038211 | 37,950 | 25·80 | 0·0 |
| Oil .. .. .. .. | 24·68 | 0·892 | 22·01 | 25 | 42·0 | 0·0965 | 0·0005057 | 49,440 | 26·46 | 7·2 |
| | | | | 35 | 34·25 | 0·1183 | 0·0007605 | 46,020 | 26·07 | 5·6 |
| | | | | 45 | 29·55 | 0·1371 | 0·0010295 | 43,710 | 25·64 | 3·9 |
| | | | | 55 | 26·0 | 0·1558 | 0·0013194 | 41,690 | 24·81 | 0·5 |
| | | | | 75 | 21·75 | 0·1863 | 0·0018850 | 39,790 | 25·12 | 1·9 |
| | | | | 95 | 18·95 | 0·2138 | 0·0024830 | 38,260 | 24·89 | 0·9 |

* Determined in an Ubbelohde viscosimeter.

TABLE V.

**RESULTS OBTAINED WITH ROTOR CONSISTING OF TWO SPHERES (PROPELLER ROTATING AT 400 R.P.M.)**

| Liquid | Viscosity* at 25°C. (cS.) | Density ($\rho$) at 25°C ($g.\ cm.^{-3}$) | Viscosity at 25°C. (cP.) | Effective Driving Weight (F) (g.) | Time for 100 Revs. (t) (sec.) | $\frac{1}{t}$ ($sec.^{-1}$) | x ($sec.^{-1}$) | Viscosity calculated from calibration curve (cS.) | % Error |
|---|---|---|---|---|---|---|---|---|---|
| Solution of Glycerine in water .. .. .. .. | 32·45 | 1·203 | 39·04 | 60 | 45·0 | 0·02222 | 0·01007 | 34·70 | 7·2 |
| | | | | 70 | 38·7 | 0·02584 | 0·00953 | 35·02 | 7·9 |
| | | | | 85 | 32·1 | 0·03115 | 0·00863 | 34·08 | 5·0 |
| | | | | 100 | 27·3 | 0·03663 | 0·00718 | 31·38 | 3·3 |
| | | | | 120 | 23·55 | 0·04246 | 0·00622 | 31·76 | 2·1 |
| | | | | 140 | 20·95 | 0·04773 | 0·00550 | 32·54 | 0·3 |
| | | | | 160 | 19·0 | 0·05263 | 0·00500 | 33·12 | 2·1 |
| Solution of Glycerine in water .. .. .. .. | 25·80 | 1·197 | 30·88 | 55 | 44·9 | 0·02227 | 0·00985 | 26·61 | 3·1 |
| | | | | 65 | 37·8 | 0·02646 | 0·00900 | 25·59 | 0·8 |
| | | | | 75 | 33·2 | 0·03012 | 0·00845 | 25·94 | 0·5 |
| | | | | 85 | 29·6 | 0·03378 | 0·00765 | 25·42 | 1·5 |
| | | | | 105 | 24·9 | 0·04016 | 0·00669 | 25·61 | 0·7 |
| | | | | 125 | 22·0 | 0·04545 | 0·00636 | 27·39 | 6·2 |
| Oil .. .. .. .. .. | 24·68 | 0·892 | 22·01 | 35 | 51·0 | 0·01961 | 0·00991 | 23·60 | 4·4 |
| | | | | 45 | 40·1 | 0·02494 | 0·00929 | 24·76 | 0·3 |
| | | | | 55 | 33·2 | 0·03012 | 0·00840 | 24·55 | 0·5 |
| | | | | 65 | 28·6 | 0·03497 | 0·00740 | 24·07 | 2·5 |
| | | | | 75 | 25·6 | 0·03906 | 0·00700 | 24·81 | 0·5 |
| | | | | 95 | 21·7 | 0·04608 | 0·00671 | 27·59 | 11·8 |
| Solution of Glycerine in water .. .. .. .. | 44·44 | 1·211 | 49·82 | 65 | 46·9 | 0·02132 | 0·01050 | 49·48 | 11·3 |
| | | | | 75 | 39·7 | 0·02519 | 0·00947 | 46·00 | 2·6 |
| | | | | 85 | 34·9 | 0·02865 | 0·00864 | 44·42 | 0·1 |
| | | | | 105 | 29·0 | 0·03448 | 0·00783 | 45·17 | 1·6 |
| | | | | 125 | 24·2 | 0·04132 | 0·00561 | 39·92 | 10·2 |

* Determined in an Ubbelohde viscosimeter.

TABLE VI.

**RESULTS OBTAINED WITH ROTOR CONSISTING OF TWO SPHERES (PROPELLER ROTATING AT 520 R.P.M.)**

| Liquid | Viscosity* at 25°C. (cS.) | Density ($\rho$) at 25°C ($g.\ cm.^{-3}$) | Viscosity at 25°C. (cP.) | Effective Driving Weight (F) (g.) | Time for 100 Revs. (t) (sec.) | $\frac{1}{t}$ ($sec.^{-1}$) | x ($sec.^{-1}$) | Viscosity calculated from calibration curve (cS.) | % Error |
|---|---|---|---|---|---|---|---|---|---|
| Oil .. .. .. .. .. | 50·09 | 0·877 | 43·93 | 65 | 41·6 | 0·02404 | 0·01361 | 50·78 | 1·4 |
| | | | | 75 | 35·0 | 0·02857 | 0·01255 | 48·73 | 2·7 |
| | | | | 85 | 31·5 | 0·03175 | 0·01253 | 52·44 | 4·7 |
| | | | | 105 | 25·1 | 0·03984 | 0·01025 | 49·28 | 1·6 |
| | | | | 125 | 31·45 | 0·04662 | 0·00908 | 49·23 | 1·7 |
| | | | | 145 | 19·05 | 0·05250 | 0·00826 | 51·02 | 1·9 |
| Solution of Glycerine in water .. .. .. .. | 46·87 | 1·212 | 56·81 | 75 | 50·9 | 0·01965 | 0·01447 | 48·77 | 4·1 |
| | | | | 95 | 36·9 | 0·02710 | 0·01236 | 43·43 | 7·3 |
| | | | | 115 | 30·9 | 0·03236 | 0·01193 | 46·46 | 0·9 |
| | | | | 135 | 26·05 | 0·03839 | 0·01026 | 44·47 | 5·1 |
| | | | | 155 | 22·7 | 0·04405 | 0·00880 | 42·55 | 9·2 |
| | | | | 175 | 20·6 | 0·04854 | 0·00831 | 44·21 | 5·7 |
| Solution of Glycerine in water .. .. .. .. | 27·34 | 1·198 | 32·75 | 65 | 46·85 | 0·02135 | 0·01365 | 25·85 | 5·4 |
| | | | | 75 | 39·7 | 0·02519 | 0·01281 | 26·01 | 4·9 |
| | | | | 85 | 35·1 | 0·02849 | 0·01257 | 27·04 | 1·1 |
| | | | | 105 | 28·8 | 0·03472 | 0·01176 | 28·42 | 4·0 |
| | | | | 125 | 24·85 | 0·04024 | 0·01116 | 30·05 | 9·9 |

* Determined in an Ubbelohde viscosimeter.

It appears from Table VII that the value of a varies both with the type of rotor and the propeller speed. The value of b, on the other hand, appears to be independent of the type of rotor but is dependent on the speed of the propeller. Both a and b decrease with decreasing propeller speed and will become zero when the propeller is stationary.

Now it has been observed that, in order to maintain the desired accuracy in determinations, the propeller speed should not be so high that appreciable agitation or disturbance of the surface of the liquid occurs. In testing suspensions it will therefore be desirable to use the lowest propeller speed that will keep the solids in homogeneous suspension, so as to avoid, as far as possible, disturbances at the surface of the liquid.

The required speed is probably dependent on the characteristics of the test fluid and may not necessarily be that for which values of a and b were determined. It would, therefore, be a distinct advantage if a relationship between propeller speed and the factors a and b could be established for every particular rotor that may be used. This matter is under investigation.

Applying equation (5) equations (3) and (4) can be written:

$$\mathbf{Re}=B\rho\left(\frac{1-b}{t}+a\right)/\eta \qquad (6)$$

$$Q=F/B^2\rho\left(\frac{1-b}{t}+a\right)^2, \qquad (7)$$

and can be used in this form to determine the viscosity of an unknown liquid in the modified apparatus provided

(a) the Q vs. **Re** relationship (or calibration curve) has been determined for the apparatus with the propeller stationary,
(b) the values of a and b have been determined for the particular test conditions,
and (c) the density of the liquid is known.

The viscosity of each liquid used in the various test series was calculated in this way using the appropriate calibration curves and values of a and b. Accepting the viscosity as determined in the Ubbelohde viscosimeter as the true value, percentage errors were calculated with the results shown in the tables. The average and maximum percentage errors for each test series are shown in Table VIII.

TABLE VIII

PERCENTAGE ERROR OF VISCOSITY DETERMINATIONS

| | Propeller Speed (r.p.m.) | | |
|---|---|---|---|
| Type of Rotor | Zero | 400 | 520 |
| Cylindrical Rotor Two Spheres | Ave. Error % 1·1 | 2·9 | 2·5 |
| | Max. Error % 4·9 | 6·8 | 7·6 |
| | Ave. Error % 3·3 | 3·6 | 4·2 |
| | Max. Error % 9·9 | 11·8 | 9·9 |

It will be observed in Table VIII that, although the introduction of a propeller to agitate the test liquid increases the error of a determination, the loss in accuracy is not appreciable. Even with the propeller stationary, discrepancies of the order of 5% for the cylindrical rotor and 10% for the other rotor are not uncommon. Such

large discrepancies are attributed mainly to mechanical features of the instrument rather than to faulty technique or calibration procedure. Despite the most careful treatment the instrument appears to be "rough" at times probably due to the entry of dust or moisture into the bearings and other moving parts. In view of these limitations a high degree of accuracy cannot be expected, particularly when small driving weights are used.

Table VIII indicates that greater accuracy is obtained with the cylindrical rotor under all test conditions.

Generally speaking, the accuracy of the method is considered to be adequate for the practical determination of the apparent viscosity of suspensions.

## APPARENT VISCOSITY OF A BARYTES SUSPENSION

While it is beyond the scope of this paper to discuss factors which influence the apparent viscosity of a suspension, brief reference may be made to the application of the suggested technique.

A suspension of 1·8 specific gravity was prepared from Barytes (s.g. 4·17) ground to pass a 200 mesh B.S. test sieve. This suspension was placed in the test cup and determinations were carried out at various speeds of the cylindrical rotor while the propeller rotated at 400 r.p.m. A fresh suspension of the same specific gravity was then prepared and the series of determinations was repeated under the same test conditions. The results of both sets of tests are reported in Table IX. The apparent viscosity values shown in the table were derived from the calibration curve (*Fig.* 2) in the manner previously described.

Since a suspension is not a true liquid it cannot have viscosity in the true sense, hence the use of the term "apparent viscosity." In effect, the apparent viscosity of a suspension may be defined as follows:

The apparent viscosity of a suspension is numerically equal to the viscosity of a hypothetical true liquid of the same density which would provide the same resistance to motion under prescribed test conditions; *i.e.* the apparent viscosity defines the "equivalent" true liquid.

It will be observed in Table IX that the results of duplicate determinations agree closely. In addition, the apparent viscosity of this particular suspension is not a constant but decreases with increasing speed of the rotor. Since the speed of the rotor is a measure of the rate of shear, this is not a surprising result for a non-ideal fluid such as a suspension.

### TABLE IX

### APPARENT VISCOSITY OF A BARYTES SUSPENSION

| Suspension | Density at 25°C. (g. cm.$^{-3}$) ($\rho$) | Effective Driving Weight (F) (g.) | Time for 100 Revs. (t) (sec.) | | Apparent viscosity as derived from calibration data. (c.S.) | |
|---|---|---|---|---|---|---|
| | | | Series 1 | Series 2 | Series 1 | Series 2 |
| —200 mesh Barytes in water | 1·8 | 29·1 | 18·45 | 18·2 | 13·42 | 12·96 |
| | | 26·1 | 22·5 | 22·6 | 15·94 | 15·94 |
| | | 24·1 | 27·35 | 27·25 | 19·97 | 19·90 |
| | | 22·1 | 35·0 | 34·9 | 24·51 | 24·43 |
| | | 20·1 | 47·35 | 47·8 | 29·29 | 29·59 |

## CONCLUSIONS

The work described indicates that it is possible to maintain a normally unstable suspension uniformly dispersed in the test cup of a Stormer viscosimeter by introducing a propeller at its base. By suitably modifying the relevant equations, it is possible to derive the viscosity of an unknown liquid from the calibration curve and test data obtained with the propeller in motion with reasonable accuracy.

It has been demonstrated that the apparent viscosity of a suspension can be readily and accurately determined.

This paper is published with the approval of the Fuel Research Board of South Africa.

Fuel Research Institute,
Pretoria.

*Received December* 19, 1953.

## REFERENCE

[1] Van der Walt, P. J. and Fourie, A. M., *J. S.A. Chem. Inst.*, 1953, **6**, 36.

# THE FLAME PHOTOMETRIC DETERMINATION OF CALCIUM IN PLANT AND BIOLOCICAL MATERIALS*

*by*

**A. STRASHEIM and J. P. NELL**

## OPSOMMING

Die belangrikste faktore wat vlamfotometriese bepalings beinvloed nagegaan. 'n Metode vir die bepaling van kalsium in plant- en biologiese materiaal word beskrywe. Die noukeurigheid waarmee die kalsiumbepalings deurgevoer kan word is ± 2%. Gelyktydig met die kalsium kan ook natrium en kalium bepaal word met dieselfde noukeurigheid as wat in die literatuur aangegee word.

## SUMMARY

An outline is given of the major interferences that are encountered during analysis with a flame photometer. A method is described for the determination of calcium in plant and biological materials. The accuracy with which calcium determinations can be made is ± 2%. Simultaneously with the calcium, sodium and potassium can be analysed with an accuracy approaching that given in the literature.

## INTRODUCTION

Flame photometric methods are now generally used when analysing for sodium, potassium and calcium in plant and biological materials. In recent publications an accuracy of 0·5 to 1% is claimed for the potassium and sodium determinations. Calcium determinations are less satisfactory and claims as regards accuracy do not exceed 3 to 4%. This paper reports an attempt to improve the accuracy with which calcium determinations can be made.

## APPARATUS

The apparatus is similar to that used by Mitchell[1]. An additional photomultiplier unit was added to facilitate the simultaneous determination of calcium, sodium and potassium with lithium as internal standard. To detect the calcium radiation at 4227 Å a R.C.A. 1P21 photomultiplier was used. The analysis lines of sodium at 5890 and 5896 Å were detected with a R.C.A. 931 A tube. To isolate the characteristic lithium radiation an Eel interference filter was used. This filter has a half bandwidth of 15 m$\mu$, and is superior to the liquid filter of Ovenston[2] which has a half bandwidth of 54 m$\mu$. With the interference filter the calcium oxide band radiation at 622 m$\mu$ is completely cut out, whereas some calcium light reaches the lithium tube when the liquid filter is used. For isolating the potassium radiation at 7665 and 7699Å the widely adopted combination of a Chance ON19 and an Ilford 207 filter proved satisfactory. Radiation on the long wavelength side is limited by the colour sensitivity of the photomultiplier. For the potassium and lithium radiations R.C.A. 1P22 photomultipliers were used.

Two types of atomisers were tested. The Leyton[3] type is preferred to the conventional Lundegardh atomiser, because of (a) the ease with which it can be operated and (b) its finer spray[4]. To prevent clogging of the very fine nozzle of the Leyton atomizer a sintered glass filter was fitted to the suction tube.

The photocurrents were observed on Cambridge spot galvonometers, the usual compensation being made to correct for background and the dark current of the photomultipliers.

Fatigue effects of the phototubes were reduced by limiting the photocurrents to less than 10$\mu$amp. with diaphragms and filters placed in the light paths, and by

* The work described in the present paper was carried out by one of us (J.P.N.) in part fullfilment of the requirements for the M.Sc. degree of the University of Pretoria.

atomising only dilute solutions. In spite of these precautions fatigue was still observed in the case of the lithium phototube. Because fatigue does not change the linear ratio that exists between photocurrent and incident light intensity deviation of the readings can be corrected for by atomising a standard solution every twenty minutes and plotting a curve of photocurrent decay against time. The actual photocurrent, in the absence of fatigue, is then easily determined.

For certain experiments Eel and Beckman flame photometers were used.

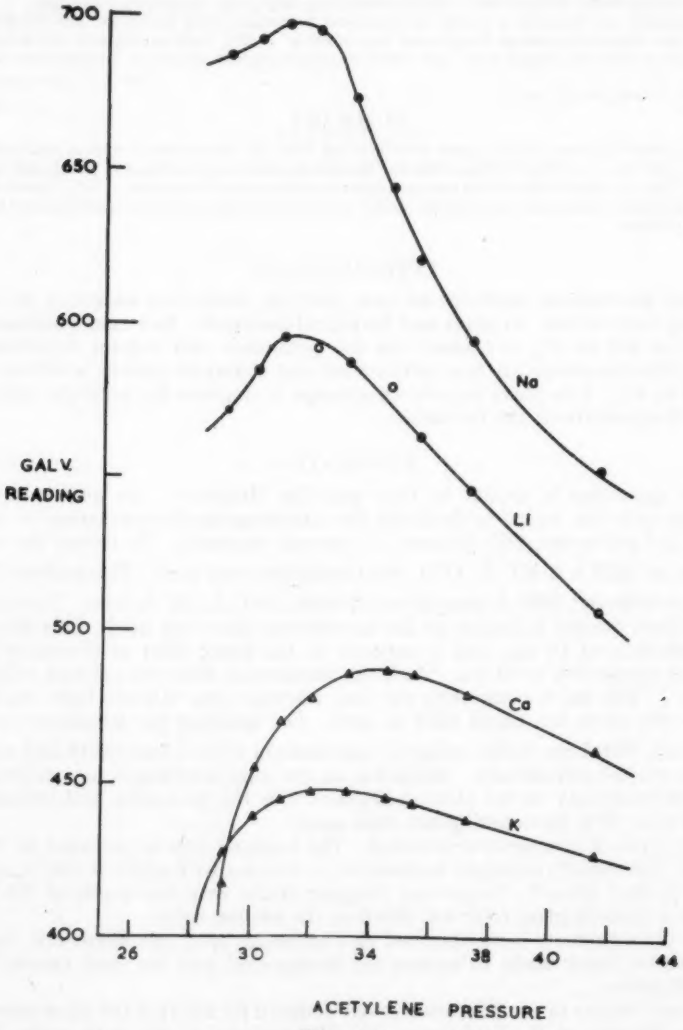



*Figure* 1

The selective excitation of calcium, sodium, potassium, and lithium, dependent on the acetylene pressure when a Leyton type of atomiser is used.

## INTERFERENCES THAT LIMIT THE ACCURACY OF ANALYSIS

**Air and acetylene pressures**

The influence of air and acetylene pressures on the excitation of the spectra is shown in *Fig.* 1. From this figure it is clear that for the ideal exitation of sodium, lithium, potassium and calcium, the acetylene pressures should be regulated to 31·4, 30·8, 32·6 and 34 cms. of water respectively. Hence it is evident the lithium is not the ideal internal standard for calcium. To avoid serious excitation differences this phenomenon demands careful adjustment of the air and acetylene pressures.

**Anionic interference**

When acids are added to the sample solutions, a suppression of the spectra of the analysis elements is observed (*Fig.* 2). It is known that the addition of various acids and salts to dilute aqueous solutions can lower the vapour pressure considerably. A remarkable resemblance, illustrated in *Figs.* 2 and 3, exists between the characteristic behaviour of the vapour pressure lowering of aqueous solutions and the anionic interference patterns.

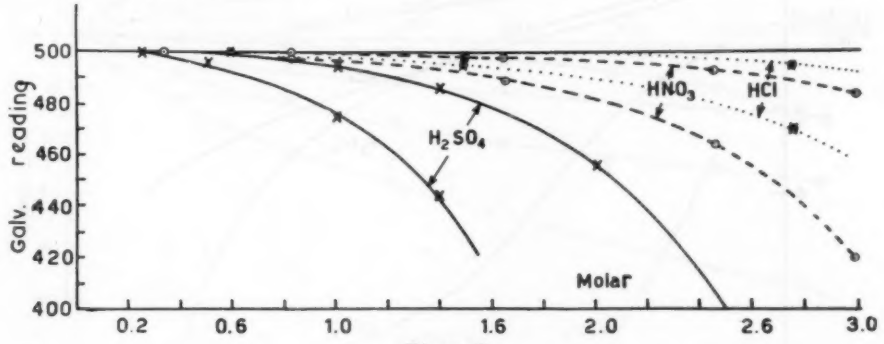



*Figure 2*

The anionic suppression of calcuim emission by $H_2SO_4$, $HNO_3$ and HCl. The upper curve in each instance was obtained when the internal standard method was employed.

---

From thermodynamic considerations it follows that

$$r = \frac{2}{p^1-p}\,\frac{Vo}{Vg-Vo} \sim \frac{2\gamma}{p^1-p}\,\frac{Vo}{Vg}$$

where

r=radius of the droplets.
$p^1$=vapour pressure in the presence of an external pressure.
p=normal vapour pressure.
$\gamma$=surface tension.
Vo=molar volume of the liquid.
Vg=molar volume of the vapour.

From this equation it is clear that the relative lowering of the vapour pressure ($p^1-p$), ($\gamma$, Vo and Vg are considered constant for dilute solutions) is inversely proportional to the size of the droplets. Small droplets will therefore evaporate more easily than larger droplets.

Considering these points it can be concluded that anionic interference can be overcome or minimised if

(i) Use is made of the internal standard principle.
(ii) An atomiser is used that gives a fine spray.
(iii) Dilute solutions are atomised to avoid excessive vapour pressure lowering of the test solutions by the various acids and salts.

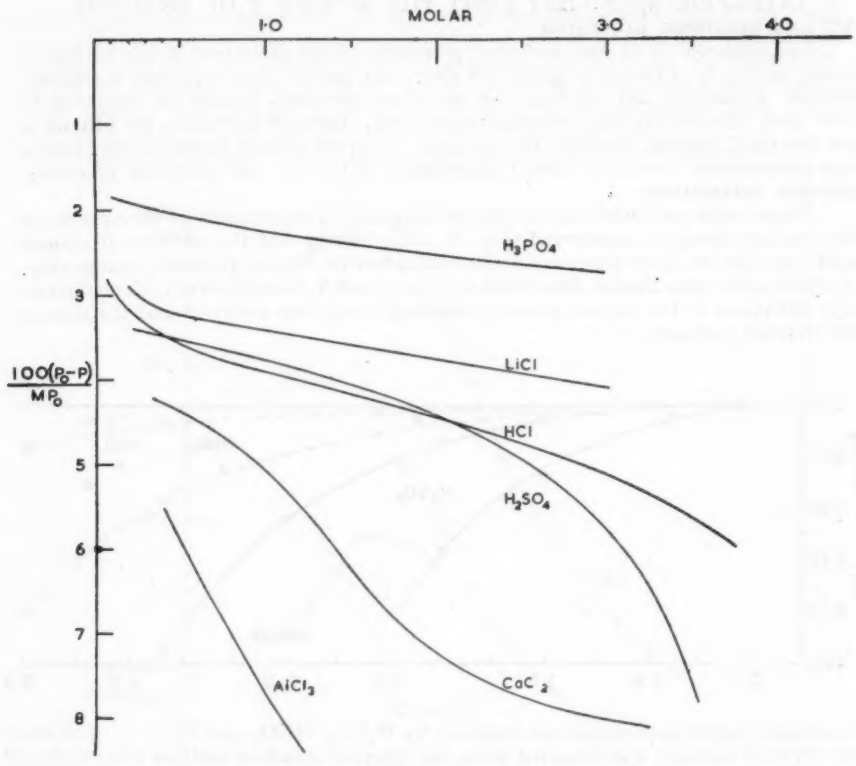



*Figure 3.*

The relative molar vapour pressure lowering of aqueous solutions by various acids and salts.

M=molecular weight of the solute.
Po=vapour pressure of solvent.
P=vapour pressure of the solution.

Graph compiled from data given in *International Critical Tables*, Vol. III, McGraw-Hill Book Company, Inc.

As the formation of the spray is also dependent on the humidity and temperature of the air used to operate the aspirator, it is advisable to atomise the samples with water saturated air.

**Extraneous element influence**

The influence of barium, zinc, iron, magnesium, aluminium, sodium and potassium on the excitation of calcium was studied. Negligible interferences were observed for barium, zinc and iron. High magnesium concentrations were found to enhance the calcium spectrum.

Appreciable amounts of sodium and potassium in the analytic solutions were found to suppress the excitation of calcium (*Fig.* 4), resulting in negative errors when determining calcium in the presence of sodium and potassium. Because the Na/Ca ratio in plant materials is usually smaller than 1/10, it follows that the sodium

content of plants will not appreciably affect the calcium determinations. The effect of potassium is more serious and can amount to 1% when calcium and potassium are present in equal amounts and to about 3% when the potassium concentration is double the calcium concentration.

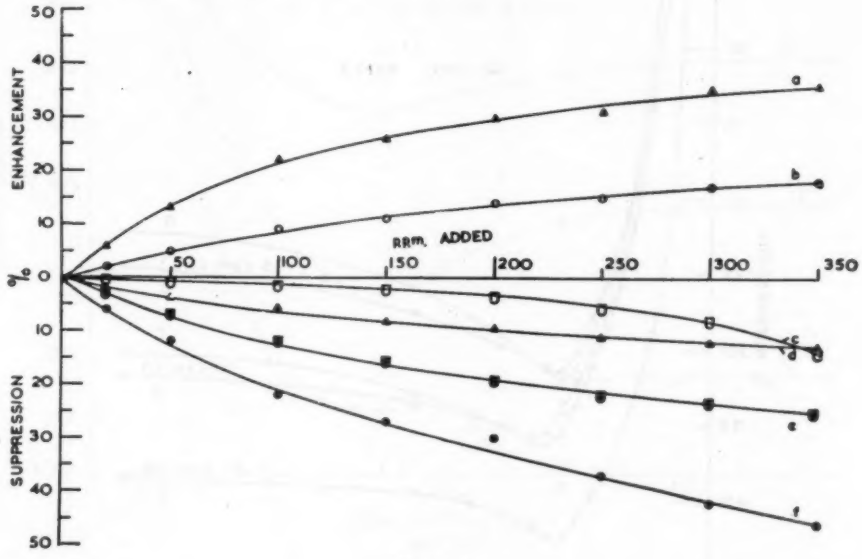



*Figure* 4.
The mutual interferences of calcium, sodium and potassium.
(a) potassium in presence of sodium;
(b) sodium in presence of potassium;
(c) calcium in presence of sodium;
(d) potassium in presence of calcium;
(e) calcium in presence of potassium;
(f) sodium in presence of calcium.

Severe suppression of the calcium spectrum was observed when aluminium or phosphorus was present in the analytical solutions. The influence of aluminium on the calcium excitation has previously been reported by Mitchell[5] who ascribed it to the lowering of the flame temperature by the addition of aluminium. The present authors could detect no interference due to aluminium when analysing for calcium in the region 0-20 p.p.m. until the Al/Ca concentration ratio exceeded 0·1. Hence in the analysis of plant material aluminium will normally not cause any interference since the Al/Ca concentration ratio in plant materials is usually less than ·05.

The influence of phosphorus on the calcium excitation was tested by adding orthophosphoric acid to the analytical solutions. The anionic interference effect of phosphorus on calcium is illustrated in *Fig.* 5. These curves differ from normal interference curves and show that the suppression of calcium is a maximum when P : Ca=1 : 2. This observed suppression must be due to occurrences in the flame since the anionic interference effect of orthophosphoric acid on the vapour pressure lowering of the samples can be neglected (see *Fig.* 3). The same phenomenon was observed when determinations were made on the calcium oxide band at 622 m$\mu$ (*Fig.* 6 (a)).

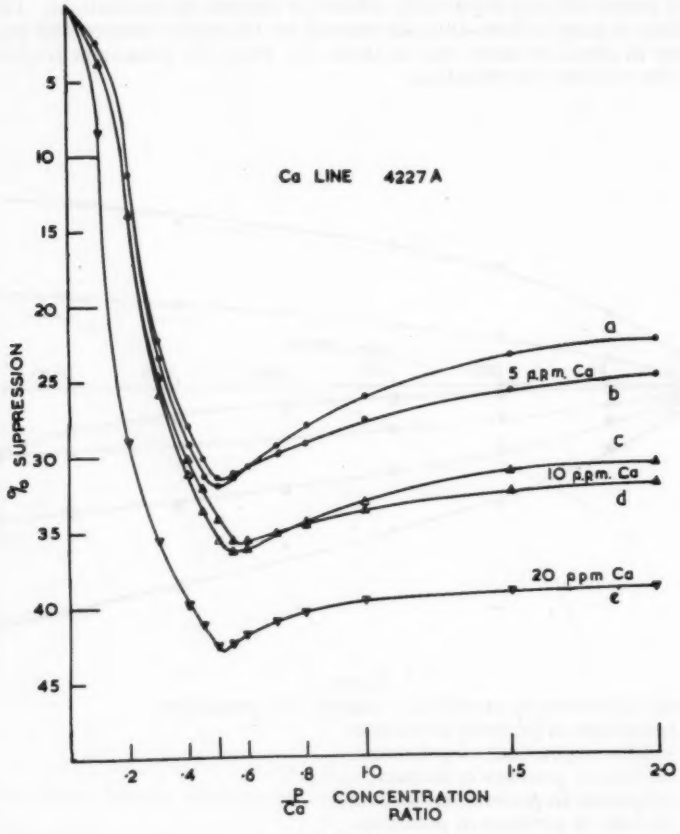



*Figure* 5.

The suppression of the calcium line at 4227Å by phosphorus.
(a) and (c) Direct readings.
(b) and (d) Internal standard readings.
(e) Internal standard and direct readings coincide.

---

The suppression of the calcium emission seems to be due to the formation of a molecule that cannot readily be excited by a flame. The $Ca_3P_2$ molecule has a P : C =1 : 1·94 ratio, but there is very little known about the properties of this compound. It is very difficult to find definite proof that the suppression is due to the formation of $Ca_3P_2$, although it seems to be the most feasible explanation, seeing that the suppression of calcium is a maximum when P : Ca=1 : 2.

In a further attempt to explain the suppression of the calcium emission by phosphorus, a number of tests were made with an Eel (butane-air flame) and a Beckman (hydrogen-oxygen flame) flame photometer. The curves illustrated in *Fig.* 6 show that the noted suppression is bigger for both the hotter (hydrogen-oxygen) and the colder (butane-air) flames than that observed for the acetylene-air flame. This finding is rather surprising and cannot be explained satisfactorily.

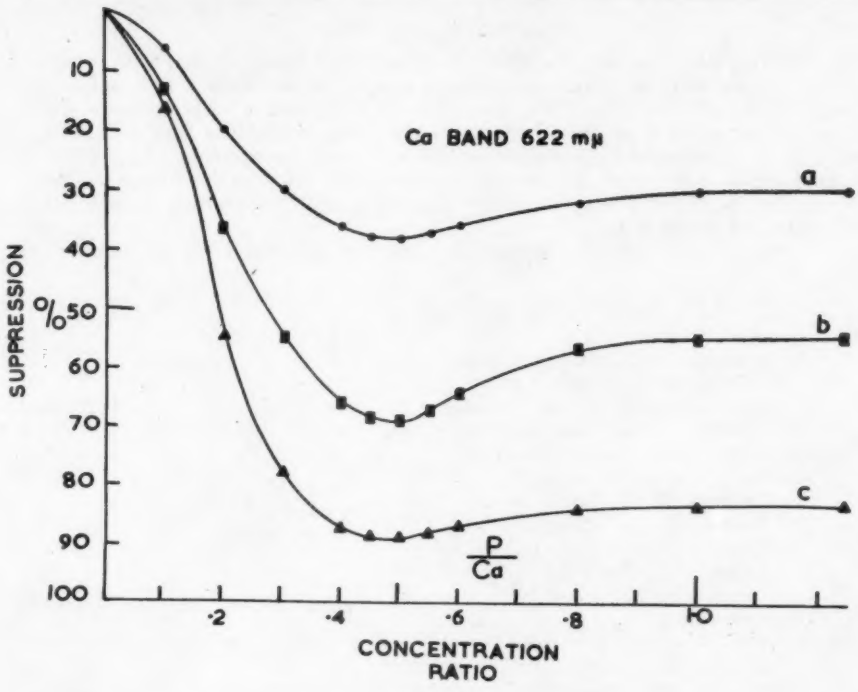



*Figure* 6.
The suppression of the calcium band at 622 mμ by phosphorus.
(a) Own flame photometer with air-acetylene flame.
(b) Beckman flame photometer with oxygen-hydrogen flame.
(c) Eel flame photometer with air-butane flame.

As previously stated, aluminium usually starts to interfere with the calcium when the Al : Ca=0·1 : 1. In the presence of an excess of phosphorus the interference is, however, negligible until this ratio has increased to about 1 : 1. When the concentration ratio P/Ca≧2, the radiation of the calcium is also not further influenced by the addition of more phosphorus. These findings suggest that for calcium analysis in the region 0 to 20 p.p.m., 50 p.p.m. phosphorus in the form of orthophosphoric acid must be added to all samples and standard solutions. In spite of the loss in sensitivity of the calcium emission (*Fig.* 5 and 6), this procedure was found to be satisfactory.*

## STANDARD SOLUTIONS

Standard solutions were prepared from Johnson and Matthey's "Specpure" chemicals. The basic standard solution was made up to contain ·1% calcium as calcium chloride, ·075% potassium as potassium chloride, and ·025% sodium as sodium chloride, and stored in a "Pyrex" bottle. Directly before use this solution is diluted ten times. The standard atomising solutions are then made up by diluting 20, 16, 12, 8, 4, and 2cc. of the ten times diluted solution to 100cc. with the addition of 5 p.p.m. lithium and 50 p.p.m. phosphorus to each.

*Since this paper was submitted for publication Baker and Johnson[7] have described a method, not substantially different from the one described in this paper, to overcome the effect of extraneous phosphorus on the emission of calcium.

## ASHING PROCEDURE

For the ashing of samples the method of Smith[6] was applied with success. To 2g. of oven dried material, until no loss in weight can be detected, 10cc. of concentrated nitric acid and 15cc. 70% perchloric acid are added. The temperature is gradually increased from room temperature to 230°C. where it is kept until all the material is digested, the solutions then having a clear straw colour. This solution is made up to 100cc. with double glass distilled water. For analysis these solutions are diluted 20, 10 and 4 times and made up to 100cc. with the addition of the internal standard and phosphorus.

The standard conditions under which analyses are carried out are summarised in Table I.

### TABLE I

*Atomiser:* Leyton type.
*Air-pressure:* 10 lb. per square inch.
*Acetylene pressure:* 32cm. of water.
*Ashing of samples:* 2g. plant material wet ashed with 15cc. 70% $HC10_4$ and 10cc. 60% $HN0_3$. These solutions are made up to 100cc. and diluted 20, 10 or 4 times to fit the standards.
*Standard Solutions:*

| | |
|---|---|
| Calcium | : 2 -20 p.p.m. |
| Potassium | : 1·5-15 p.p.m. |
| Sodium | : ·5-5 p.p.m. |
| Lithium | : 5 p.p.m. |
| Phosphorus | : 50 p.p.m. |

*Analysis lines:*

| *Element* | *Wavelength of emitted light* | *Excitation potential* | *Phototube* | *How isolated* |
|---|---|---|---|---|
| Potassium | 7665-7699Å | 1·61eV | R.C.A. 1P 22 | Chance ON19+ Ilford 207 filters |
| Lithium | 6708Å | 1·84eV | R.C.A. 1P 22 | Lithium interference filter |
| Sodium | 5890-5896Å | 2·10eV | R.C.A. 931A | With spectrometer |
| Calcium | 4227Å | 2·92eV | R.C.A. 1P 21 | With spectrometer |

## SENSITIVITY AND ACCURACY

The absolute sensitivity of detection of calcium is $\pm$·25 p.p.m. For accurate analyses it is, however, not advisable to determine calcium concentrations lower than 2 p.p.m. in the final analytical solutions.

Representative data revealing the accuracy obtainable when analysing for calcium are given in Tables II and III. Four portions of two plant samples, X and Y, were individually wet-washed, made up to 100cc. and analysed flame photometrically adding excess phosphorus (50 p.p.m.) to the final analytical solutions.

The results tabulated in Table II give the variation of the analytic values from the mean average, while the results tabulated in Table III compare chemical and flame photometric values determined for a series of plant samples. To eliminate weighing errors, due to the known varying hygroscopic nature of plant materials, the different samples for the chemical and flame photometric analyses were weighed out simultaneously.

From the results tabulated in Tables II and III it can be concluded that:

(1) The internal standard flame photometric values can be repeated with an accuracy of $\pm 1\%$.
(2) The direct flame photometric values can be repeated with an average accuracy of $\pm 2{\cdot}0\%$.
(3) The internal standard flame photometric values agree to within 2% with the chemical values, determined by using the standard oxalate method.
(4) The direct flame photometric values do not agree too well with the chemical values.

TABLE II

| Sample | Flame Photometric Determinations % Ca | | Sample | Flame Photometric Determinations % Ca | |
|---|---|---|---|---|---|
| | Direct | Internal Standard | | Direct | Internal Standard |
| X1 | 2·62* | 2·61 | Y1 | 1·81 | 1·82 |
| | 2·63 | 2·61 | | 1·81 | 1·82 |
| | 2·63 | 2·60 | | 1·81 | 1·82 |
| X2 | 2·64 | 2·62 | Y2 | 1·82 | 1·82 |
| | 2·65 | 2·62 | | 1·83 | 1·83 |
| | 2·65 | 2·62 | | 1·82 | 1·83 |
| X3 | 2·69 | 2·66 | Y3 | 1·87 | 1·86 |
| | 2·70 | 2·66 | | 1·87 | 1·86 |
| | 2·70 | 2·66 | | 1·88 | 1·86 |
| X4 | 2·74 | 2·67 | Y4 | 1·88 | 1·86 |
| | 2·74 | 2·68 | | 1·87 | 1·85 |
| | 2·75 | 2·67 | | 1·87 | 1·85 |
| Av. | 2·68 | 2·64 | | 1·84 | 1·84 |
| % Dev. † | 18·4 | 1·14 | | 1·64 | 0·96 |

*Three repeat determinations using same test solution.

$$\dagger\% \text{ Deviation} = \frac{\delta}{\bar{x}} \times 100$$

$$\delta = \frac{\sqrt{\Sigma\, d^2}}{n-1}$$

and $d = \bar{x} - x$ or $x - \bar{x}$.

## APPLICATIONS

### Fishmeal

The Ca/P ratio in fishmeal, containing calcium as calcium phosphate, is ideal for maximum depression of the calcium excitation by phosphorus. Results in Table IV show that a 40% error was made in the flame-photometric determination of the calcium in fishmeal when no compensation for the phosphorus content of the samples was made.

TABLE III

| SAMPLES | FLAME PHOTOMETRIC % Ca | | PERCENTAGE STANDARD DEVIATIONS OF THE FLAME PHOTOMETRIC VALUES | | CHEMICAL* % Ca | PERCENTAGE STANDARD DEVIATIONS BETWEEN DIRECT FLAME PHOTOMETRIC AND CHEMICAL VALUES | PERCENTAGE STANDARD DEVIATIONS BETWEEN INTERNAL FLAME PHOTOMETRIC AND CHEMICAL VALUES |
|---|---|---|---|---|---|---|---|
| | Direct | Internal Standard | Direct | Internal Standard | | | |
| A | 2·49 | 2·43 | 5·0 | ·37 | 2·39 | 4·17 | 1·67 |
| B | 1·37 | 1·48 | 1·69 | ·84 | 1·47 | 7·28 | ·68 |
| C | 1·45 | 1·36 | 2·97 | ·84 | 1·35 | 7·4 | ·74 |
| D | 4·40 | 4·55 | 6·93 | 1·29 | 4·44 | ·91 | 2·48 |
| E | 2·45 | 2·59 | 3·42 | 1·66 | 2·56 | 3·52 | 1·16 |
| F | 2·05 | 2·11 | 4·44 | 1·62 | 2·21 | 7·24 | 4·52 |
| G | 1·96 | 2·04 | 3·70 | 1·65 | 2·10 | 6·7 | 2·86 |
| Average | | | 4·02 | 1·18 | | 5·32 | 2·01 |

*The chemical determinations were made according to the standard oxalate method.

TABLE IV

| SAMPLE | Flame Photometric Determinations %Ca | | Chemical Determinations %Ca | |
|---|---|---|---|---|
| | P compensated for | P not compensated | C.S.I.R.* | F.I.R.I.† |
| Pilchard meal | 5·89 | 3·60 | 5·5 | 5·73 |
| Maasbanker meal | 5·22 | 3·25 | 5·4 | 5·58 |

*C.S.I.R.: Council for Scientific and Industrial Research.
†F.I.R.I.: Fishing Industry Research Institute.

**Plant Material**

Sodium and potassium can also be analysed in the analytical solutions prepared for the analysis of calcium. In Table V comparative data of calcium, potassium and sodium analyses of various types of plant material are given. Two sets of data are given—one set was obtained using the method described above, and the other by a method in use at the Division of Chemical Services.*

TABLE V

Flame Photometric Analyses

| SAMPLES | C.S.I.R. | | | CHEMICAL SERVICES* | | |
|---|---|---|---|---|---|---|
| | % Ca | % K | % Na | % Ca | % K | % Na |
| Peach Leaves .. .. | 2·66 | 2·63 | ·059 | 2·75 | 2·67 | ·050 |
| Bean Leaves .. .. | 1·84 | 1·61 | ·050 | 1·80 | 1·50 | ·050 |
| Little Gem Leaves .. | 3·79 | 2·47 | ·062 | 3·75 | 2·42 | ·063 |
| Plum Leaves .. .. | 1·33 | 1·32 | ·044 | 1·20 | 1·24 | ·055 |
| Lucern .. .. .. | 1·48 | 1·57 | ·330 | 1·00 | 1·40 | ·330 |

The results tabulated in Table V show that comparable flame photometric results can be obtained even when one set of analyses is made according to the direct analytical technique.

ACKNOWLEDGEMENTS

The authors wish to thank the Division of Chemical Services of the Department of Agriculture and Dr. Haul and Miss Rethemeyer of the National Chemical Research Laboratory for chemical analyses. This paper is published with the permission of the South African Council for Scientific and Industrial Research, Pretoria.

National Physical Laboratory,
South African Council for Scientific and Industrial Research,
Pretoria. *Received February* 10, 1954.

REFERENCES

1 Mitchell, R. L., *Spectrochim. Acta*, 1950, **4**, 62.
2 Ovenston, T. C. J. and Robinson, A. M., *Analyst*, 1951, **76**, 416.
3 Leyton, L., *Analyst*, 1951, **76**, 723.
4 Nell, J. P., Thesis: "Die vlamfotometriese bepaling van Natrium, Kalium en Kalsium in plantmateriaal", Pretoria University, 1953.
5 Mitchell, R. L., *J. Soc. Chem. Ind.*, 1936, **55**, 269.
6 Smith, G. F., *Analyt. Chim. Acta*, 1953, **8**, 397.
7 Baker, G. L. and Johnson, L. H., *Anal. Chem.*, 1954, **26**, 465.

*At the Chemical Services a Beckman Flame Photometer attachment without an internal standard control is used. Results taken from Report No. 292/52 of the Division of Chemical Services.

# THE COMPOSITION OF THE BASES FROM AN ISCOR COAL TAR

*by*
**F. W. HOUGEN and (Mrs.) M. A. RAAL**

OPSOMMING

Mengsels van YSKOR koolteerbasisse is deur middel van distillasie en infrarooi-spektroskopie kwantitatief ontleed. Elf verskillende basisse is bepaal.

SUMMARY

Mixtures of bases from an ISCOR coal tar have been quantitatively analysed for their contents of eleven component bases by a method of distillation and infra-red spectroscopy.

Three separate mixtures of coal tar bases obtained from the high temperature carbonisation of coal at the South African Iron and Steel Industrial Corporation Limited (ISCOR Works, Pretoria) have been examined. It was considered desirable to investigate this South African material in order to make information as to its composition available.

The major constituents of coal tar bases have long been known, and some are being produced commercially. They find uses in the pharmaceutical, fine chemical and dyestuff industries[1].

Quantitative separation of individual bases in tar base mixtures is not practicable because of the similar chemical and physical properties of many of the bases. Fractional distillation will effect the isolation of pyridine, $\alpha$-picoline and the greater part of the quinoline, whilst the remaining bases are obtained in groups containing three or more constituents having similar boiling points.

Various methods of limited applicability have been advanced for the quantitative analysis of tar base mixtures. 2:6-Lutidine, and $\beta$- and $\gamma$-picolines have been estimated with the aid of their ultra-violet absorption spectra[2], and the latter two bases can also be estimated by a colorimetric method[3]. The quantitative separation of the $\beta$- and $\gamma$-picolines and of the 2-methyl- and 8-methylquinolines has been effected by counter-current solvent distribution[4]. A method has also been described[5] whereby the mixed picolines and lutidines are oxidised with selenium dioxide to carboxylic acids which are then estimated by paper chromatography. A most satisfactory general method has been reported by Coulson *et al.*[6, 7], in which bases that cannot be separated by distillation are estimated by their infra-red absorption spectra. Coulson reports the estimation of picolines, lutidines and collidines by this technique.

Coulson's method was adopted in the present work, and also successfully extended to a higher boiling fraction containing quinoline, isoquinoline and quinaldine. The bases investigated were obtained from a high temperature carbonisation of blended South African coals in coke ovens of the Becker type. Coals from the Durban Navigation Colliery at Dannhauser in Natal and from the Blesbok Colliery and the Navigation Colliery of the South African Coal Estates (both these latter in the Witbank area) were blended in roughly equal proportions. Bases were extracted from the Once Run Benzole, the Once Run Heavy Solvent Naphtha and the Tar Distillates. The three base mixtures, referred to as ORB, ORHS and TD, were separately investigated.

After a rough preliminary distillation, the bases were fractionated at about 650 mm. pressure through a column which had an efficiency of about 40 theoretical plates. The course of the distillations is shown in Fig. 1 where distillation temperature is plotted against weight of accumulated distillate. The weights are expressed as a percentage of the total so as to facilitate the making of comparisons between the three base mixtures. The distillates were collected in small fractions for later analysis.

Fractions which were not accounted for by analysis are indicated in Fig. 1 by the broken lines.

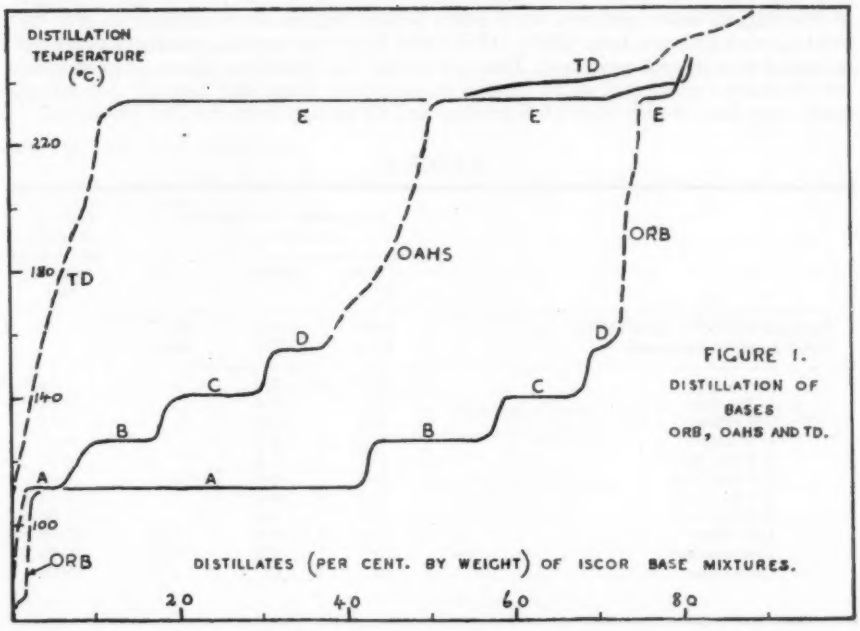



FIGURE 1.
DISTILLATION OF BASES ORB, OAHS AND TD.

The boiling temperatures of the distillation plateaux suggested the following qualitative composition:—

| | | | |
|---|---|---|---|
| Plateau | A | b.p. | 112-113°:pyridine. |
| ,, | B | ,, | 126-127°:α-picoline. |
| ,, | C | ,, | 140-141°:β- and γ-picolines, 2:6-lutidine. |
| ,, | D | ,, | 155-156°:2:3-, 2:4- and 2:5-lutidines, 6-ethyl-2-methylpyridine. |
| ,, | E | ,, | 234-236°:quinoline. |

A qualitative examination by infra-red spectroscopy of one fraction from each distillation plateau proved the composition to be as suggested except that 6-ethyl-2-methylpyridine was absent. Pyrrole, which occurs in small amount in the "α-picoline" fraction of coal tars[8, 9], was not detected in this fraction with Ehrlich's reagent (p-dimethylaminobenzaldehyde), nor by its infra-red spectrum. Fractions in the boiling range 236-250° were found to consist of quinoline, isoquinoline and quinaldine. 8-Methylquinoline, which has the same boiling point as quinaldine (247°/760 mm.), was not detected.

Pure specimens of all components (standards) were required for reference in the infra-red analysis, and the following were prepared: pyridine, α-; β- and γ-picolines, 2:3-, 2:4-, and 2:6-lutidines, quinoline, isoquinoline and quinaldine. Pure 2:5-lutidine was presented by Dr. E. A. Coulson.

Quantitative analyses with the aid of infra-red spectroscopy were carried out on all distillation fractions in the boiling ranges 128-160° (including plateaux C and D) and 230-250° (including plateau E). Fractions in the boiling range 110-128° (including plateaux A and B) were more conveniently analysed by their refractive indices since not more than two components (pyridine and α-picoline) were present.

Fractions not accounted for by analysis were those boiling below 110°, between 160° and 230° and above 250°. The analytical results are summarised in Table I. Columns 2, 3 and 4 give the contents of individual bases expressed as percentage by weight of the original base mixtures. In Column 5 these figures are combined to give the total amount of bases from ORB, ORHS and TD, expressed in gallons of bases per thousand tons of coal processed. This calculation was based on figures of production per thousand tons of coal of 2·5 gallons of bases from Once Run Benzole, 1·4 gallons from Once Run Heavy Solvent Naphtha, and 85 gallons from the Tar Distillates.

TABLE I

| | Percentage composition of base mixtures | | | Gallons of bases per 1000 tons of coal |
|---|---|---|---|---|
| | ORB | ORHS | TD | |
| Recovered after 2 distillations .. .. .. | 80·8 | 81·2 | 88·7 | |
| Total material analysed .. .. .. .. | 75·0 | 72·4 | 66·3 | |
| Pyridine .. .. .. .. .. .. | 39·8 | 7·6 | | 1·10 |
| α-Picoline .. .. .. .. .. .. | 15·9 | 10·0 | | 0·54 |
| β-Picoline .. .. .. .. .. .. | 3·7 | 3·9 | | 0·15 |
| γ-Picoline .. .. .. .. .. .. | 2·8 | 2·2 | | 0·10 |
| 2-6-Lutidine .. .. .. .. .. | 3·7 | 5·4 | | 0·17 |
| 2:3-Lutidine .. .. .. .. .. | 0·5 | 0·9 | | 0·03 |
| 2:4-Lutidine .. .. .. .. .. | 2·1 | 4·5 | | 0·12 |
| 2:5-Lutidine .. .. .. .. .. | 1·1 | 2·4 | | 0·06 |
| Quinoline .. .. .. .. .. .. | 4·4 | 28·1 | 43·8 | 37·70 |
| Isoquinoline .. .. .. .. .. | 0·1 | 0·5 | 12·2 | 10·40 |
| Quinaldine .. .. .. .. .. .. | 0 | 0 | 7·1 | 6·04 |

## EXPERIMENTAL

Unless otherwise stated, all distillations were carried out at about 650 mm. pressure (atmospheric in Pretoria) in a column (91 × 1·2 cm.) packed with Dixon rings (1/16 in. × 1/16 in.; 100 × 100 mesh Staybrite) and equivalent to about 40 theoretical plates under total reflux.

### Preparation of standards

A standard was considered as pure when other bases could not be detected in its infra-red absorption spectrum.

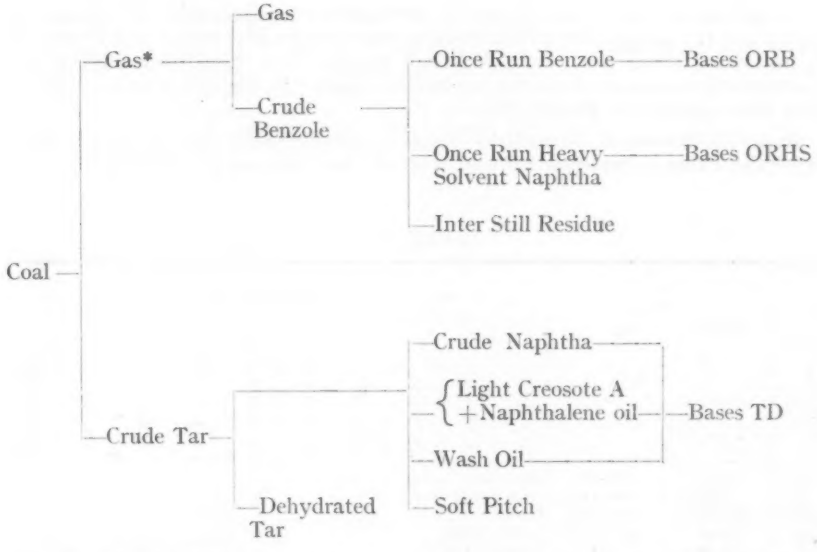
Six bases were obtained by distillation of purchased reagents at a reflux ratio of 1:40, viz.:

pyridine, b.p. 112·5°, $n_D^{25}$1·5065; α-picoline, b.p. 126·3°, $n_D^{25}$1·4981; β-picoline, b.p. 141°, $n_D^{25}$1·5036; quinoline, b.p. 235°, $n_D^{25}$1·6223; isoquinoline, b.p. 241°, $n_D^{25}$1·6205; quinaldine, b.p. 246°, $n_D^{25}$1·6092. Purchased samples of γ-picoline and 2:6-lutidine were purified by repeated crystallisations at low temperature[9] until constant freezing points were obtained, viz.: γ-picoline, f.p. +3·4°, $n_D^{25}$1·5011; 2:6-lutidine, f.p. −7·8°, $n_D^{25}$1·4950. Purchased "2:4-lutidine" was found by infra-red inspection to contain a considerable amount of 2:3-lutidine. "2:4-Lutidine" was distilled at a reflux ratio of 1:30. A fraction (b.p. 156°, $n_D^{25}$1·4978) enriched in 2:4-lutidine and another fraction (b.p. 156-159°, $n_D^{25}$1·5004) enriched in 2:3-lutidine were taken as starting materials for the isolation of these two bases. 2:3-Lutidine was isolated via its addition com-

pound with urea[10]. Three crystallisations of the urea adduct, followed by steam distillation, benzene extraction of the distillate and fractionation of the benzene extract through a short column yielded 2:3-lutidine (b·p. 153-154°, $n_D^{25}$1·5045). 2:4-Lutidine was isolated via its hydrochloride[6]. The hydrocholride, crystallised from benzene/ethanol, melted at 118-121°: addition of KOH to the hydrochloride caused 2:4-lutidine ($n_D^{25}$1·4971) to separate out as a liquid phase.

**Source of base mixtures**

The subdivision of products from the coal high temperature carbonisation is shown diagrammatically below.




* The gas is scrubbed with wash oil, obtained by distillation of the tar.

**Distillation of base mixtures**

The ISCOR batches (ORB, ORHS, TD) in weighed aliquots (391 g., 304 g., 928 g.) were dried over solid KOH. The decanted bases (379 g., 298 g., 926 g.) were distilled at a reflux ratio of 1:3. The distillates (373 g., 287 g., 905 g.), after being kept over solid KOH, were refractionated at a reflux ratio of 1:50. The distillates (316 g., 247 g., 823 g.) were collected in small fractions (respectively 31, 46 and 24 in number) which were weighed and kept over solid KOH until analysed.

**Infra-red analysis**

The spectra were recorded in carbon disulphide solution with a Perkin-Elmer Infra-red Spectrometer Model 12C; rock salt prism; path length 0·5 mm. It was possible to select an absorption peak for each standard at a wavelength where negligible absorption occurred from any other base of similar boiling point. Thus the following wavelengths were selected for analysis: pyridine, 9·37$\mu$; $\alpha$-picoline, 9·08$\mu$ and 7·73$\mu$; $\beta$-picoline, 8·90$\mu$; $\gamma$-picoline, 9·35$\mu$; 2:3-lutidine, 12·77$\mu$; 2:4-lutidine,

13·25$\mu$; 2:5-lutidine, 8·01$\mu$; 2:6-lutidine, 8·65$\mu$ and 7·90$\mu$; quinoline, 13·17$\mu$; isoquinoline, 10·81$\mu$; quinaldine, 13·00$\mu$. Calibration curves of optical density against concentration were made for the standards at the selected wavelengths, and the "base line technique"[11] was used for the analysis.

The accuracy of the analyses was such that the estimated amounts of individual bases in a fraction added up to more than 95% of the total fraction. If required, a higher accuracy could have been obtained by adjusting the concentrations of samples to be measured for infra-red absorption to the most ideal values in all cases.

**Refractive index analysis**

An Abbé refractometer was used.

A calibration curve was prepared for different compositions of pyridine/α-picoline, and the composition of fractions on and between plateaux A and B (Fig. 1) were estimated using this curve. Although samples were kept over solid KOH, refractive indices were not strictly reproducible, apparently because of rapid moisture uptake after opening the sample tubes.

As an illustration of the method, detailed experimental results for Batch ORB are given in Table II. Summarised results for all three batches are in Table I.

TABLE II

| ORB Fraction No. | Weight | b.p., °C. | Composition (in g.) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pyridine | α-Picoline | β-Picoline | γ-Picoline | 2:6-Lutidine | 2:3-Lutidine | 2:4-Lutidine | 2:5-Lutidine | Quinoline | Isoquinoline |
| 1 | 6·33 | 65–78 | Not | analy | sed. | | | | | | | |
| 2 | 3·87 | 78–110 | Not | analy | sed. | | | | | | | |
| 3–15 | 149·00 | 110–113 | 149·0 | | | | | | | | | |
| 16 | 15·40 | 113–127 | 5·4 | 10·0 | | | | | | | | |
| 17 | 17·49 | 127 | 1·0 | 11·5 | | | | | | | | |
| 18 | 7·65 | 127 | | 7·7 | | | | | | | | |
| 19 | 11·38 | 127 | | 11·4 | | | | | | | | |
| 20 | 8·99 | 127 | | 9·0 | | | | | | | | |
| 21 | 10·35 | 127–131 | | 10·1 | | | | | | | | |
| 22 | 12·85 | 131–141 | | 2·4 | 3·7 | 1·3 | 5·0 | | | | | |
| 23 | 8·61 | 141 | | 0·1 | 3·0 | 1·9 | 3·4 | | | | | |
| 24 | 10·95 | 141 | | | 4·1 | 2·7 | 4·0 | | | | | |
| 25 | 9·86 | 141–145 | | | 3·7 | 3·5 | 2·1 | | | | | |
| 26 | 12·21 | 145–158 | | | | 1·3 | | 0·9 | 6·1 | 3·9 | | |
| 27 | 3·84 | 158–162 | | | | | | 0·9 | 1·9 | 0·5 | | |
| 28 | 13·09 | 162–236 | Not | analy | sed. | | | | | | | |
| 29 | 8·41 | 236 | | | | | | | | | 7·3 | |
| 30 | 7·91 | 236–242 | | | | | | | | | 7·6 | |
| 31 | 3·16 | 242–250 | | | | | | | | | 2·4 | 0·3 |
| | | Total | 155·4 | 62·2 | 14·5 | 10·7 | 14·5 | 1·8 | 8·0 | 4·4 | 17·3 | 0·3 |

The work reported here is a continuation of earlier experiments carried out by Mr. C. A. J. Hoeve.

The authors thank Dr. D. A. Sutton for his interest in the work, the Scientific and Research Department at ISCOR, Pretoria, for providing the coal tar bases and the South African Bureau of Standards for the use of their infra-red spectrometer. This paper is published by permission of the South African Council for Scientific and Industrial Research.

National Chemical Research Laboratory,
C.S.I.R., Pretoria.

*Received February* 25, 1954.

## REFERENCES

[1] Boyle, J. L., *Ind. Chem. Chem. Manuf.*, 1953, **29**, 251; Kruber, O., *Angew. Chem.*, 1949, **61**, 59.
[2] Herington, E. F. G., *Discuss. Faraday Soc.*, 1950, No. 9, 26.
[3] Herington, E. F. G., *Analyst*, 1951, **76**, 90; Ballard, C. W. and Scott, P. G. W., *Chem. and Ind.*, 1952, 715.
[4] Columbic, C., *Analyt. Chem.*, 1952, **24**, 1849.
[5] Jerchel, D. and Jacobs, W., *Angew. Chem.*, 1953, **65**, 342.
[6] Coulson, E. A., Hales, J. L., Holt, E. C. and Ditcham, J. B., *J. Appl. Chem.*, 1952, **2**, 71.
[7] Coulson, E. A. and Hales, J. L., *Analyst*, 1953, **78**, 114.
[8] Densham, A. B., Langston, D. J. and Gough, G., *J.*, 1952, 2433.
[9] Coulson, E. A. and Jones, J. I., *J. Soc. Chem. Ind.*, 1946, **65**, 169.
[10] Milner, D. W. and Yorkshire Tar Distillers, B.P. 584, 148, (1947).
[11] Wright, N., *Ind. Eng. Chem. Anal.*, 1941, **13**, 1.

# A RELATIONSHIP BETWEEN SUTHERLAND'S CONSTANT AND THE MAXIMUM ENERGY OF ATTRACTION BETWEEN GAS MOLECULES

*by*

**C. J. G. RAW**

OPSOMMING

Van beskikbare gegewens vir twee-en-twintig verskillende gasse word 'n empiriese vergelyking, $\epsilon/\mathbf{k} \sim 0{\cdot}88$ C, tussen die intermolekulere kragkonstante, $\epsilon/\mathbf{k}$, en Sutherland se konstante C, afgelei. Benaderde verhoudings tussen Sutherland se konstante en ander eienskappe word bespreek.

SUMMARY

From available data for twenty-two different gases, an empirical relation, $\epsilon/\mathbf{k} \sim 0{\cdot}88$ C. between the inter-molecular force constant, $\epsilon/\mathbf{k}$, and Sutherland's constant C, is obtained, Approximate relations between Sutherland's constant and other properties are discussed.

Probably the most useful expression for the potential energy of molecular interaction is the Lennard-Jones (12,6) model for which the potential energy is expressed as a function of the molecular separation and two intermolecular force constants, $\epsilon$, the maximum energy of attraction between two molecules, and $r_0$, the collision diameter for low-energy head-on collisions. These constants can be obtained from gas viscosity data by the method of Hirschfelder, Bird, and Spotz[1] (the HBS method). This method is extremely laborious, and it is of value to have some simple and rapid means of obtaining a good approximation to the intermolecular force constant, $\epsilon/\mathbf{k}$, ($\mathbf{k}$=Boltzmann's constant).

If the experimental values[2,3] of Sutherland's constant, C (which expresses the temperature dependence of the gas viscosity), are plotted against values[4,5] of $\epsilon/\mathbf{k}$ computed by the HBS method, a straight line passing through the origin is obtained. Experimental data for twenty-two different gases have been used in constructing this plot (shown on the accompanying graph). It is clear that a simple proportionality

$$\epsilon/\mathbf{k} \sim 0{\cdot}88\mathrm{C} \qquad (1)$$

exists between C and $\epsilon/\mathbf{k}$.

The relationship is not obeyed for hydrogen and helium, the two gases of lowest molecular weight. In the case of carbon monoxide, krypton, carbon dioxide, and chlorine, which do not appear to obey (1), a much better fit is obtained (see accompanying graph) if $\epsilon/\mathbf{k}$ values from critical temperature data[2] replace those determined by the HBS method[4]. The method of obtaining $\epsilon/\mathbf{k}$ from the critical temperature, $T_c$, depends on a relation from the Lennard-Jones and Devonshire theory of gases and liquids[6]. This theory provides several relationships between $\epsilon/\mathbf{k}$ and other properties which can be related to Sutherland's constant by using (1). Three such relationships are:

$$\epsilon/\mathbf{k} \sim 0{\cdot}75T_c \qquad (2)$$

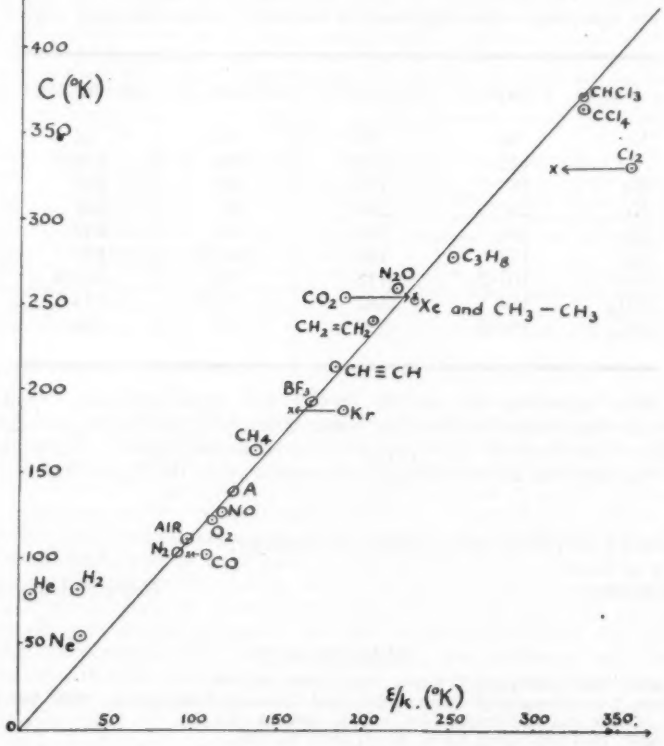
$$\epsilon/\mathbf{k} \sim 1{\cdot}39T_b \qquad (3)$$

$$\text{and } \Delta H_e \sim 0{\cdot}678z\epsilon\mathbf{N} = 0{\cdot}678 \times 11 \times \epsilon/\mathbf{k} \times \mathbf{R} \qquad (4)$$

where $T_c$ is the critical temperature, $T_b$ is the boiling point, $\Delta H_e$ is the molar latent heat of vaporisation, $\mathbf{N}$ is Avogadro's number, $\mathbf{R}$ is the molar gas constant, and $z$ is the number of nearest neighbours of a given molecule. Lark-Horowitz[6], in an experimental study of the scattering of X-rays by liquids, has shown that the average value of $z$ is about 11 for normal liquids.

PLOT OF SUTHERLAND'S CONSTANT AGAINST $\epsilon/k$.

$\odot \equiv \epsilon/k$ from viscosity data by the HBS method.
$\times \equiv \epsilon/k$ from critical temperatures.



Using equations (2), (3), (4) in conjunction with (1), the following relationships between C and T*c*, T*b*, and $\Delta He$ are obtained:

$$C \sim 0{\cdot}85 T_c \qquad (5)$$

$$C \sim 1.58 T_b \qquad (6)$$

$$\Delta He \sim 0{\cdot}01314\ C \text{ kcal. mole}^{-1}. \qquad (7)$$

Equations (5) and (6) are in good agreement with two empirical rules proposed by Rankine[9] and Vogel[10]:

$$C \sim 0.89 T_c \qquad \text{(Rankine)}$$

$$\text{and } C \sim 1{\cdot}47 T_b \qquad \text{(Vogel)}$$

This agreement with independently proposed empirical equations supports the accuracy and usefulness of equation (1).

Values of C calculated by (5), (6), (7) from experimental T*c*, T*b*, and $\Delta He$ data[2, 7, 8] are compared with experimental values[2, 3] in the following table:

| Gas | C(expt.) | C(from T*c*) | C(from T*b*) | C(from $\Delta He$) |
|---|---|---|---|---|
| Ne | 56 | 38 | 43·2 | 34 |
| A | 142 | 133·5 | 138 | 119·5 |
| Kr | 188 | 178 | 190 | 165 |
| Xe | 252 | 246 | 262 | 231 |
| $N_2$ | 104 | 107 | 122 | 102 |
| $O_2$ | 125 | 131 | 142·5 | 125 |
| CO | 101·2 | 113 | 128 | 110·5 |
| $CH_4$ | 164 | 162 | 175·3 | 155·5 |
| $BF_3$ | 194·6 | 222 | 272 | 309 |

The three equations are seen to provide fair approximations. Equation (7) appears to be most unsatisfactory for boron trifluoride, but this is probably due to the fact that (7) as deduced above, applies only to normal liquids. This lends support to other evidence that boron trifluoride is associated in the liquid state.

Department of Chemistry and Chemical Technology,
University of Natal,
Pietermaritzburg.

*Received March* 19, 1954.

## REFERENCES

[1] Hirschfelder, Bird and Spotz, *J. Chem. Phys.*, 1948, **16**, 968.
[2] Partington, "An Advanced Treatise on Physical Chemistry", Longmans, 1949, Vol. I.
[3] For $BF_3$: Cooke and Mackenzie, *This Journal*, 1951, **4**, 123.
[4] Hirschfelder, Bird and Spotz, *Chem. Revs.*, 1949, **44**, 205.
[5] For $BF_3$: Raw, *This Journal*, 1954, **7**, 20.
[6] As given in: Fowler and Guggenheim, "Statistical Thermodynamics", C.U.P., 1939.
[7] As given in: Guggenheim: "Thermodynamics", North Holland Publishing Co., 1950.
[8] For $BF_3$: Booth and Martin, "Boron Trifluoride and its Derivatives", Wiley, 1949.
[9] Rankine: *Proc. Roy. Soc.*, 1910, **84**, 181.
[10] Vogel: *Ann. Phys.*, 1954, **43**, 1235.

# CHEMISTRY OF NARAS SEED (*ACANTHOSICYOS HORRIDA*, HOOK)

## PART I—A PHYSICO-CHEMICAL STUDY OF THE PROTEINS FROM NARAS SEED

*by*

**F. J. JOUBERT and D. C. COOPER**

OPSOMMING

'n Fisies-chemiese studie van die eiwitte bevat in narassaad (*Acanthosicyos horrida*, Hook-is onderneem. Deur gebruik te maak van die ligverstrooiingsmetode word 'n gemiddelde molekulêrgewig van 377,000 vir die meer onoplosbare eiwit gevind, teenoor 'n baie kleiner molekulêrgewig van 58,000 vir die meer oplosbare eiwit. Eersgenoemde waarde word deur ultrasentrifuge-metings bevestig. Diffusie-metings toon aan dat die laasgenoemde eiwit nie homogeen is nie, maar 'n hoe graad van homogeniteit word vir eersgenoemde gevind.

Narassaad-eiwit is in buffers van lae ioniese sterkte feitlik onoplosbaar. Dit los egter geredelik op in buffers wat natriumdodesielsulfaat bevat, wat aantoon dat 'n eiwit-natriumdodesielsulfaat kompleks gevorm word. Die molekulêrgewigte van die kompleks is baie kleiner as die van die oorspronklike eiwit, bv. met ioniese sterktes van 0·1, pH = 7·8 word 'n molekulêrgewig van 88,200 gevind.

SUMMARY

A physico-chemical study has been made of the proteins from naras seed (*Acanthosicyos horrida*, Hook). The molecular weight by the light scattering method gives an average value of 377,000 for the main, more insoluble fraction as against a much lower molecular weight of 58,000 for the more soluble fraction. The former value is confirmed by ultracentrifugal measurement. Diffusion measurements indicate some degree of heterogeneity for the latter fraction, but a high degree of homogeneity is found for the former.

Whereas naras seed protein is practically insoluble in low ionic strength buffers, it dissolves readily in the same buffer containing sodium dodecyl sulphate, which indicates that a protein-detergent complex is formed. The molecular weight of the complex is very much lower than that of the original protein, e.g. at I = 0·1, pH = 7·8, a molecular weight of 88,200 is found.

The naras (*Acanthosicyos horrida*, Hook) is a cucurbitaceous plant, which occurs particularly near Walvis Bay in South West Africa. The fruit is a small melon consisting of an outer rind and six segments which contain numerous seeds. The fruits and the seeds derived from them were extensively used as foods by the desert dwellers of the area in earlier days, and are still used to some extent in this way. More recently, the seeds have been collected and exported to South Africa where they have found use in the confectionary industry. Versfeld and Britten[1] found that the seed kernels consist mainly of protein (35%) and fat (53%). Preliminary results on naras seed proteins have already been published[2].

A large number of seed globulins has been studied by ultracentrifuge techniques[3-6] and electrophoretic examination was carried out on some of them[7-9]. Apart from ultracentrifugal methods, the light scattering method has also been used[10-12] for the molecular weight determination of seed globulins, with results in good agreement with those by the ultracentrifuge. As an ultracentrifuge only became available after most of the present work was completed, reliance was placed on the light scattering method. This has been repeatedly shown to be reliable for proteins[13], provided sufficient care has been taken in preparation of solutions and in calibration of the photometer to absolute standards. In addition to molecular weight determinations of naras seed proteins, in ordinary buffers and in sodium dodecyl sulphate solutions, measurements have also been made of diffusion.

## METHODS

A Brice-Phoenix light scattering photometer[14] was used for the measurement of light scattered at 45°, 90° and 135° to the incident beam. The blue mercury line of wave length 4358 Å and the green 5461 Å were used for turbidity measurements. The solu-

tions were prepared dust free by centrifugation at *ca.* 20,000 r.p.m. for 30 minutes and a final ultrafiltration through nitrocellulose membranes as described by Goring and Johnson[15]. Using these methods, dissymetry values $\left(\frac{I_{45}}{I_{135}}\right)$ between 1·05 and 1·08 were obtained. It was assumed that these were residual effects resulting from forward scatter by a very small amount of high scattering material which has escaped removal during preparation of the solution. For reasons outlined in a previous publication[16], the 90° scatter was not corrected for dissymmetry. Benzene, a standard sample of polystyrene and Ludox solutions were used for absolute calibration of light scattered intensities at 90° as described[16].

Protein concentrations were determined by micro-Kjeldahl, the nitrogen content of naras seed proteins having been determined as 18·12±0·10%. Refractive index increments were measured using a modified Pulfrich refractometer with a divided cell. Refractive index increments were measured at 5780 A and values of 0·196 (concentration in g./c.c.) at 4358 A and 0·188 at 5461 A were calculated by using Perlmann and Longsworth[17] equation.

Diffusion coefficients have been determined at 20°C. by the free boundary method using the Tiselius U tube for boundary formation. A plunger type of compensator was used to move the boundary between protein and buffer solution into the observation channel without disturbances.

## PREPARATION OF PROTEINS

Naras seed was defatted by repeated extraction with pentane. The proteins of oil-free naras seed meal were extracted with four separate volumes of 10% sodium chloride over a period of 24 hours at 30°C. The final saline extract was centrifuged at 2500 r.p.m. and further cleaned by filtration through a pad of kieselguhr. The proteins were fractionated as outlined in *Fig.* 1.

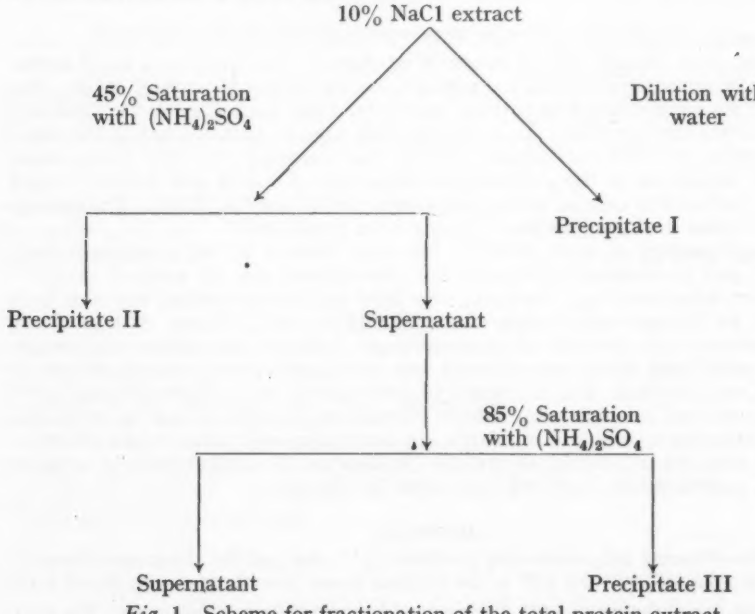



*Fig.* 1. Scheme for fractionation of the total protein extract

Precipitate I was obtained by the slow addition of four volumes of water to each volume of the 10% sodium chloride extract, causing precipitation of the extracted protein. This was allowed to stand overnight at 5°C. The precipitate recovered by centrifugation in the cold was redissolved in 10% sodium chloride and reprecipitated as before. The final precipitate, washed free from chloride with cold water, was freeze dried.

To the 10% sodium chloride extract saturated ammonium sulphate was added with stirring to 45% saturation and the resulting precipitate (II) was collected by centrifugation. The precipitate was redissolved in 10% sodium chloride and reprecipitated by the addition of saturated ammonium sulphate. Precipitate III was obtained from the supernatant of precipitate II by further addition of solid ammonium sulphate to 85% saturation. This precipitate, after redissolving in 45% saturated ammonium sulphate, was reprecipitated at 85% saturation. Both precipitates II and III were stored under their respective mother liquors at 5°C. Precipitates II and III were present in the total protein extract approximately in the ratio 5 : 1 respectively.

## EXAMINATION OF NARAS SEED PROTEINS IN BUFFERS OF HIGH SALT CONTENT

It has been observed that the extracted protein (precipitates I and II) is practically insoluble in low ionic strength buffers at room temperature, e.g., buffers of ionic strength (I) of 0·1 and pH 8 to 9. To get some information about the solubility, the solubility of precipitate I was determined in different concentrations of sodium chloride solution (*Fig.* 2). The solutions were allowed to stand in contact with an excess of protein overnight at 20°C. and then clarified by centrifugation for 10 minutes at 20,000 r.p.m. Concentrations were determined by micro-Kjeldahl. The solubility is small up to 0·66 M sodium chloride, but above this concentration the solubility increases rapidly.

In Table I the light scattered molecular weights found for the preparations of *Fig.* 1 under different conditions of pH values and ionic strengths are given and a typical graph of $\frac{c}{\gamma}$ against c is shown in *Fig.* 3.

TABLE I

**LIGHT SCATTERING MOLECULAR WEIGHTS OF DIFFERENT NARAS SEED PROTEIN FRACTIONS**

| Preparation | I | pH | Molecular Weight | | |
|---|---|---|---|---|---|
| | | | 4358 A | 5461 A | Average |
| Precipitate I .. .. .. .. | 1·0 | 7·90 | 370,000 | 388,000 | 379,000 |
| Precipitate II .. .. .. | 1·0 | 6·51 | 375,000 | 391,000 | 383,000 |
| | 1·0 | 7·90 | 361,000 | 375,000 | 368,000 |
| | 1·0 | 8·64 | 371,000 | 387,000 | 379,000 |
| | 1·0 | 10·02 | 188,000 | 193,000 | 191,000 |
| | 0·7 | 7·90 | 381,000 | 392,000 | 387,000 |
| | 0·5 | 7·91 | 361,000 | 378,000 | 370,000 |
| Precipitate III .. .. ... | 1·0 | 6·44 | 58,100 | 57,800 | 58,000 |
| | 0·7 | 7·82 | 59,900 | 58,500 | 59,200 |

It is noted that the molecular weight of precipitate I is about the same as that of precipitate II. Thus, the two preparations probably represent the same protein fraction. At I=1·0, pH=10·02, a definitely lower molecular weight was obtained for

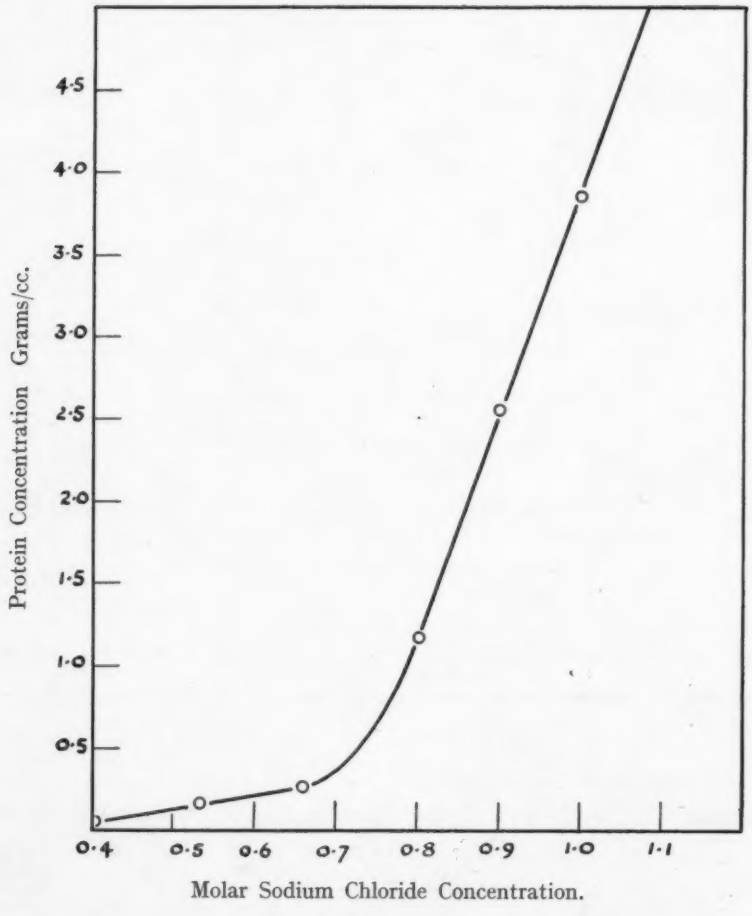



Fig. 2 Solubility of Naras Seed Protein (Precipitate I) in Sodium Chloride Solutions.

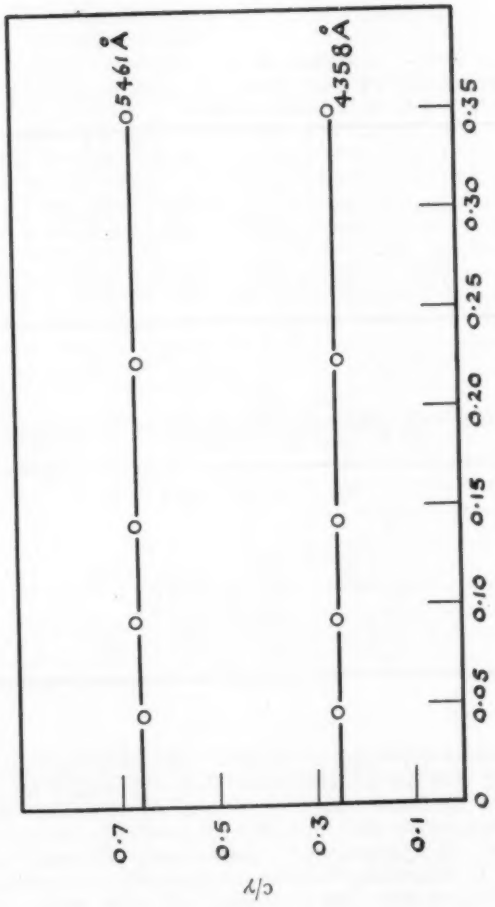



Fig. 3. c/γ against c for Naras Seed Protein (Precipitate I) in buffer I = 1·0, pH = 7·90.

precipitate II. This pH value probably falls outside the pH stability range of the protein and the lower molecular weight indicate sthat the protein is broken into smaller units. The molecular weight of precipitate III was very much lower than that of the first fraction.

In Tables II and III diffusion coefficients determined at 20°C. in borate buffer of I=1·0, pH=8·6, calculated by the maximum height, $D^{\circ}_{20}$ ($H_m$), and the standard deviation, $D^{\circ}_{20}$ ($\sigma^2$), methods for precipitates II and III, are listed.

TABLE II

**DIFFUSION COEFFICIENTS OF PRECIPITATE II IN BORATE BUFFER,**
I=1.0, pH=8.6 at 20°C.

| Diffusion time (hr.) | 8·53 | 19·77 | 23·70 | 28·45 | 33·30 | 44·07 | Average |
|---|---|---|---|---|---|---|---|
| $D^{\circ}_{20}$ ($H_m$) × 10⁻⁷ cm.²/sec. | 3·09 | 3·07 | 3·15 | 3·06 | 3·13 | 3·23 | |
| | — | 3·03 | 3·12 | 3·05 | 2·98 | 3·11 | 3·09 |
| $D^{\circ}_{20}$ ($\sigma^2$) × 10⁻⁷ cm.²/sec. | 3·11 | 3·24 | 3·23 | 3·07 | 3·17 | 3·17 | |
| | 3·11 | 3·20 | 3·13 | 3·19 | 3·11 | 3·14 | 3·16 |

TABLE III

**DIFFUSION COEFFICIENTS OF PRECIPITATE III IN BORATE BUFFER,**
I=1.0, pH=8.6 at 20°C.

| Diffusion time (hr.) | 7·05 | 18·05 | 22·10 | 26·23 | 33·18 | Average |
|---|---|---|---|---|---|---|
| $D^{\circ}_{20}$ ($H_m$) × 10⁻⁷ cm.²/sec. | 6·83 | 6·95 | 6·90 | 6·99 | 7·04 | |
| | 6·49 | 6·76 | 6·83 | 6·79 | 6·72 | 6·83 |
| $D^{\circ}_{20}$ ($\sigma^2$) × 10⁻⁷ cm.²/sec. | 8·26 | 8·23 | 8·39 | 8·17 | 8·21 | |
| | 8·02 | 8·17 | 8·27 | 8·21 | — | 8·21 |

The diffusion coefficients of precipitate III were very much higher than that of precipitate II, indicating therefore a much higher molecular weight for precipitate II in accordance with the light scattering observations. Whereas the two methods of calculation gave approximately the same diffusion coefficient for precipitate II, indicating a high degree of homogeneity[18], considerably different values were obtained for precipitate III, suggesting therefore a certain degree of heterogeneity[18]. From the diffusion coefficient and light scattering molecular weight a frictional ratio of 1·40 was calculated for precipitate II.

After the above work was completed a Spinco electrically driven ultracentrifuge was installed, and a brief examination of precipitate II was carried out on this. Precipitate II showed a major component of sedimentation constant 12·6 Svedberg Units (S.U.), and a small quantity *ca.* 5% of a fast component. Using these sedimentation data and previously determined diffusion coefficient the molecular weight given in Table IV was found. The molecular weight and frictional ratio agree very well with the light scattering values.

TABLE IV

SEDIMENTATION MOLECULAR WEIGHT OF PRECIPITATE II

| | |
|---|---|
| $D^{\circ}_{20}$ cm.$^2$/sec. .. .. .. | $3{\cdot}15 \times 10^{-7}$ |
| $S^{\circ}_{20}$ Svedberg Units .. .. | 12·6 |
| Partial specific volume .. | 0·74 |
| Molecular weight .. .. | 373,000 |
| Frictional ratio .. .. .. | 1·41 |

## EXAMINATION OF NARAS SEED PROTEIN (PRECIPITATE I) IN SODIUM DODECYL SULPHATE SOLUTIONS

Whereas naras seed protein was practically insoluble in phosphate buffer of I=0·1, pH=7·8 at room temperature, it was observed that the protein dissolved quite readily in the same buffer containing sodium dodecyl sulphate (S.D.S.). This fact alone seems to indicate that a protein-detergent complex has been formed.

The composition of the complex was estimated and it was found that the average detergent uptake at I=0·1, pH=7·8 was 0·47 gram detergent per gram of protein. The refractive index values of the complexes were lower than those found for the protein. For a complex prepared at I=0·1, pH=7·8 a value of 0·164 was found at 5780 A and values of 0·165 at 5461 A and 0·171 at 4358 A were calculated by using Perlmann and Longsworth[17] equation.

The light scattering molecular weight found for complex prepared at I=0·1, pH=7·8 was 88,200. The molecular weight of this complex is very much lower than that of the original protein, hence the protein was significantly broken down in complex formation. A further study of naras protein-detergent complexes is in hand.

## DISCUSSION

In Weissberger[19] (p. 704, Table VII) the more recent molecular data on proteins have been assembled. From Weissberger the data on seed globulins have been selected and are shown in Table V. In this table the light scattering molecular weight of the seed globulins has been entered, as well as the sedimentation molecular weight of edestin obtained by Polson[20].

TABLE V

**MOLECULAR CONSTANTS OF SEED GLOBULINS**

| Protein | $V_{20}$ | $S_{20}$ | $D_{20}$ | $M_S$ | M (Light scattering) | $\frac{f}{f_0}$ |
|---|---|---|---|---|---|---|
| Excelsin .. .. .. | 0·743 | 13·3 | 4·26 | 295,000 | 279,000[21] | 1·13 |
| Legumin .. .. .. | 0·73 | 12·6 | 3·5 | 330,000 | 346,000[10] | 1·33 |
| Amandin .. .. .. | 0·746 | 12·5 | 3·45 | 346,000 | — | 1·32 |
| Edestin .. .. .. | 0·744 | 12·8 | 3·18 | 381,000 | 335,000[11] | 1·39 |
| Edestin (Polson)[20] .. | 0·744 | 12·8 | 3·93 | 309,000 | 330,000[10] | 1·21 |
| Naras seed protein .. | (0·74) | — | 3·15 | — | 377,000 | 1·40 |

The molecular weight obtained for naras seed protein (precipitate I, entered in Table V) is closely similar to the molecular weight of edestin reported by Weissberger, though somewhat higher than the light scattering molecular weight of edestin and the value reported by Polson. Further it is noticed that the diffusion coefficient

and fractional ratio for naras seed protein are also close to the values reported for edestin by Weissberger. In comparing the solubility of naras seed protein in sodium chloride solutions (*Fig.* 1) with that of edestin[3], it is noticed that, though naras seed protein is more soluble, it resembles the solubility of edestin in requiring a high salt concentration.

Although it is known that a more soluble protein fraction is associated with seed globulins, this has only been studied in detail by Johnson and Naismith[22] in the case of groundnut protein. Our results seem to confirm Johnson and Naismith's findings with respect to the heterogeneous nature of this fraction.

Whereas it was possible to dissolve naras seed proteins in buffers of low ionic strength with the aid of S.D.S., the low molecular weight of the resulting complex compared with that of the original protein, indicates that considerable disaggregation and probably severe structural changes of the protein have occurred. The addition of S.D.S. to arachin in solution[23] causes a similar breakdown of a high molecular weight protein.

Calculations from the known composition of the complex at I=0·1, pH=7·8 have shown that a complex has been formed in which the weight of protein per molecule of complex is about 59,000. This suggests breakdown of the original protein molecule into sixths.

One of us (D.R.C.) wishes to express his appreciation to the Leather Industries Research Institute, South Africa, for a scholarship. To Dr. P. C. Carman we are greatly indebted for his interest and criticism at all times. This paper is published by permission of the South African Council for Scientific and Industrial Research.

National Chemical Research Laboratory,
C.S.I.R., Pretoria.

*Received June* 10, 1954.

## REFERENCES

[1] Versfeld, W. and Britten, G., *Report of the S.A. Association for the Advancement of Science.* Pretoria, 1915.
[2] Joubert, F. J. and Cooper, D. R., *Nature*, 1953, **172,** 1190.
[3] Svedberg, T. and Stamm, A., *J. Amer. Chem. Soc.*, 1929, **51,** 2170.
[4] Svedberg, T. and Sjögren, B., *J. Amer. Chem. Soc.*, 1930, **52,** 279.
[5] Johnson, P., *Trans. Farad. Soc.*, 1945, **42,** 28 and 36.
[6] Danielsson, C. E., *Biochem. J.*, 1949, **44,** 387.
[7] Irving, G. W. and Fontaine, T. D., *Arch. Biochem.*, 1945, **7,** 475.
[8] Fontaine, T. D. and Irving, G. W., *Arch. Biochem.*, 1945, **8,** 239.
[9] Johnson, P. and Shooter, E. M., *Bioch. Biophys. Acta*, 1950, **5,** 361 and 376.
[10] Goring, D. and Johnson, P., *Trans. Farad. Soc.*, 1952, **48,** 367.
[11] Beeckmans, M. L. and Lontie, R., *Bull. Soc. Chim. biol.*, 1946, **28,** 509.
[12] Putzeys, P. and Beeckmans, M. L., *Bull. Soc. Chim. biol.*, 1946, **28,** 503.
[13] Doty, P. and Edsall, J. T., *Advances in Protein Chemistry*, 1951, VI, 35.
[14] Brice, B., Halwer, M. and Speiser, R., *J. Opt. Soc. Amer.*, 1950, **40,** 768.
[15] Goring, D. and Johnson, P., *J. Chem. Soc.*, 1952, 33.
[16] Joubert, F. J (to be published)
[17] Perlmann, G. E. and Longsworth, L. G., *J. Amer. Chem. Soc.*, 1948, **70,** 2719.
[18] Charlwood, P. A., *J. Phys. Chem.*, 1953, **57,** 125.
[19] Weissberger, A., *Physical Methods of Organic Chemistry*, Vol. I, Part 1, *Interscience Publishers*, 1949, 706.
[20] Polson, A., *Kolloid-Z.*, 1939, **87,** 149.
[21] Putzeys, P. and Brosteaux, J., *Mededeel. Koninkl. Vlaam. Acad. Wetenschap., Belg. Klasse Wetenschap*, 1941, 3, No. 1.
[22] Johnson, P. and Naismith, W. E. F., *Discussions Farad. Soc.*, 1953, No. 13, 98.
[23] Johnson, P. and Joubert, F. J., *J. Polymer Sci.*, 1951, **7,** 605.

# ELECTROPHORETIC PROPERTIES AND MOLECULAR WEIGHTS OF ACACIA PLANT GUMS

*by*

**F. J. JOUBERT**

OPSOMMING

Die elektroforetiese eienskappe van *cyanophylla*—gom en akasiagom is bestudeer. Mobiliteite vir akasiagom is altyd kleiner as die van *cyanophylla*-gom. 'n Osmotiesedruk-molekulêrgewig van 142,000 word vir *cyanophylla*-gom gevind. Alhoewel vir *cyanophylla* gom alleen een komponent met elektroforese waargeneem word, toon diffusie-metings dat die gom nie homogeen is nie.

SUMMARY

The electrophoretic behaviour of *cyanophylla* gum and gum arabic has been studied. Gum arabic mobilities are always less than those of *cyanophylla* gum. A number average molecular weight of 142,000 is found for *cyanophylla* gum. Though single boundaries are observed in electrophoresis for *cyanophylla* gum, diffusion measurements show a certain degree of hetetogeneity.

Electrophoretic examinations of various polysaccharides have been reported. Four samples of carrageenin, ranging in intrinsic viscosity from 3·4 to 11·2, were examined electrophoretically by Cook, Rosé and Colvin[1]. The patterns obtained showed no definite separation in components, but considerable boundary spreading was noticed. Colvin, Cook and Adams[2] studied electrophoretically various heterogeneous polysaccharides and polysaccharide mixtures in molar potassium hydroxide. No clear separation of the components in any of these cases was observed. The present paper describes an electrophoretic examination of two plant gums, gum arabic and *cyanophylla* gum (*Acacia cyanophylla*). As these were readily soluble in ordinary buffers, it was possible to examine them under normal electrophoretic conditions. Measurements of the molecular weight and of the diffusion coefficient of *cyanophylla* gum were also made.

## EXPERIMENTAL

### Methods

Electrophoresis was carried out in a Tiselius electrophoretic apparatus manufactured by Hilger Watts Ltd., using a medium U-tube equipped with a long centre section. Dialysis of the gum solution was usually performed in a slowly rotating cellophane bag against a large volume of the required buffer solution. Dialysis was carried out for 24 hours with 3 or 4 changes of buffer. All electrophoretic measurements were made at 1°C.

Diffusion coefficients have been determined by the free boundary method using the Tiselius U-tube for boundary formation. A plunger type of compensator was used to move the boundary between gum and buffer solution into the observation channel without disturbances.

Number average molecular weights were determined by osmotic pressure measurements at 7·5°C using Adair[3] osmometers.

A Brice-Phoenix light scattering photometer described by Brice, Halwer and Speiser[4] was used for the measurement of weight average molecular weights.

### Materials

The present sample of *cyanophylla* gum was collected from a single species (*Acacia cyanophylla*) of a group of acacias commonly known as Port Jackson willow

by Dr. A. M. Stephen of the University of Cape Town, who kindly supplied a purified specimen. Purification was effected by the methods of Smith [5].

The crude gum (100 grams) was dissolved in water (300c.c.) giving a dark brown, neutral solution which was filtered through a layer of cellulose powder. The filtrate was acidified with 2N hydrochloric acid (40c.c.) and the gum precipitated by pouring the acidified solution into ethanol (1500c.c.). Two further such precipitations were carried out and finally an aqueous solution of gum acid was poured into ethanol. The purified material was washed with acetone and dried in vacuum (20mm.) at 45°C. for 30 hours, yielding a white amorphous powder (65 grams).

Gum arabic is usually a mixture of acacia gums, predominantly from *Acacia arabica*. The present sample was a commercial sample supplied by Eimer and Amend, New York.

## RESULTS

### Electrophoresis

The electrophoretic diagrams obtained when *cyanophylla* gum at a concentration of 1·63% was examined at different pH values and constant ionic strength (I) of 0·15 are shown in Fig. 1. The descending boundaries showed considerable spreading, whilst the ascending boundaries remained very sharp. The $\delta$ and $\epsilon$ boundaries, at the initial position of the boundaries, were also of considerable size. These observations, together with the difference between ascending and descending boundary mobilities (Table I), indicate extensive boundary anomaly effects.

TABLE I

MOBILITIES OF *CYANOPHYLLA* GUM IN BUFFERS OF I=0·15 AND DIFFERENT pH VALUES AT 1°C. GUM CONCENTRATION = 1·63%

| pH | Mobility × $10^{-4}$ cm.$^2$ sec.$^{-1}$ volt$^{-1}$ | | |
|---|---|---|---|
| | Ascending | Descending | Difference |
| 7·62 | −0·94 | −0·71 | 0·23 |
| 5·46 | −0·91 | −0·71 | 0·20 |
| 2·88 | −0·65 | −0·50 | 0·15 |

To reduce these effects, the next series of experiments was carried out at a lower gum concentration (0·66%) and the ionic strength of the buffers was increased to 0·2. The difference between ascending and descending boundary mobilities was smaller (Table II) and slightly better electrophoretic patterns (Fig. 2) were obtained, but ideal electrophoresis was not achieved.

This electrophoretic behaviour is surprising, since in all the measurements the gum solution was dialysed against buffer solvent until dialytic equilibrium was established and pH and conductivity measurements (Table III) on dialysed gum solution and buffer solvent gave normal values. The conductivity of the buffer is *ca.* 1·5% higher than that of the gum solution, but this is to be expected as a result of the Donnan distribution of diffusible ions over the membrane used for dialysis.

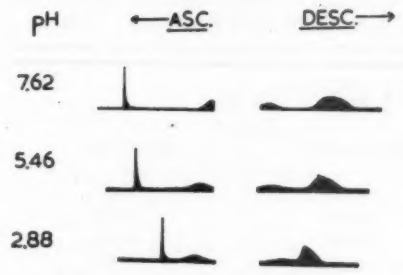



*Fig.* 1.—Electrophoretic diagrams of *cyanophylla* gum in buffers of I=0·15 at different pH values at 1°C. Gum concentration=1·63%.

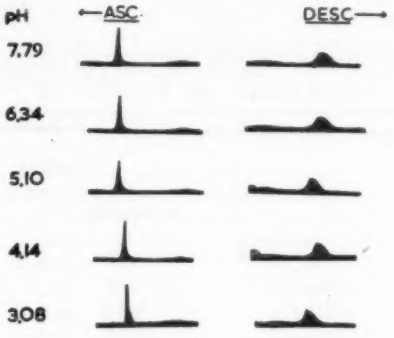



*Fig.* 2.—Electrophoretic diagrams of *cyanophylla* gum in buffers of I=0·20 at different pH values at 1°C. Gum concentration=0·66%.

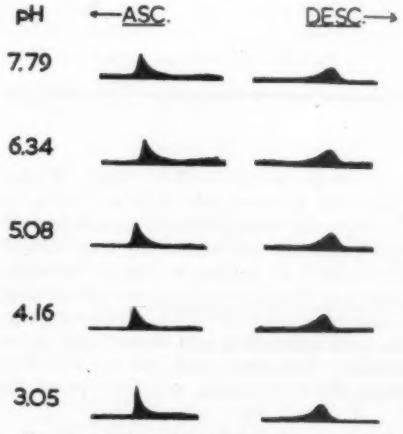



*Fig.* 3.—Electrophoretic diagrams of gum arabic in buffers of I=0·20 at different pH values at 1°C. Gum concentration=0·66%.

*Fig.* 4.—Electrophoretic diagrams of *cyanophylla* gum and gum arabic mixture in buffer of I=0·2, pH=7·8 at 1°C.

TABLE II

MOBILITIES OF *CYANOPHYLLA* GUM IN BUFFERS OF I=0·20 AND DIFFERENT pH VALUES AT 1°C. GUM CONCENTRATION = 0·66%

| pH | Mobility $\times 10^{-4}$ cm.$^2$ sec.$^{-1}$ volt$^{-1}$ | | |
|---|---|---|---|
| | Ascending | Descending | Difference |
| 7·79 | −0·70 | −0·63 | 0·07 |
| 6·34 | −0·69 | −0·62 | 0·07 |
| 5·10 | −0·64 | −0·60 | 0·04 |
| 4·14 | −0·62 | −0·56 | 0·06 |
| 3·08 | −0·48 | −0·43 | 0·05 |

TABLE III

pH VALUES AND CONDUCTIVITIES OF *CYANOPHYLLA* GUM SOLUTIONS AT 1°C. GUM CONCENTRATION = 0·66%

| pH | | Conductivities (mhos) | | |
|---|---|---|---|---|
| Buffer | Gum solution | Buffer | Gum solution | % difference |
| 7·84 | 7·79 | $8·73 \times 10^{-4}$ | $8·59 \times 10^{-4}$ | 1·6 |
| 6·35 | 6·34 | 8·56 | 8·45 | 1·3 |
| 5·13 | 5·10 | 9·21 | 9·07 | 1·5 |
| 4·18 | 4·14 | 9·03 | 8·91 | 1·3 |
| 3·11 | 3·08 | 9·37 | 9·21 | 1·7 |

The gum was negatively charged in the pH range (3·08—7·79) of study. Whilst the electrophoretic mobility was not much affected between pH 7·79 and 4·14, it was considerably less at pH 3·08, indicating that this pH value produces a considerable decrease in the electrolytic dissociation of the gum. As electrophoresis was carried out under conditions such as to eliminate boundary anomalies as far as possible, it must be concluded that the observed differences between ascending and descending boundary patterns under these conditions are due to a property of the dissolved gum. In spite of the single boundaries observed on both ascending and descending sides it was felt that the spreading of the descending boundary and the considerable difference between descending and ascending electrophoretic patterns must be attributed to electrophoretic heterogeneity.

Similar measurements were carried out on gum arabic at a concentration of 0·66% (Fig. 3). In this case, the descending and ascending patterns, although rather asymmetric, approximate more closely mirror images and the difference between ascending and descending boundary mobilities is much smaller. (Table IV).

TABLE IV

MOBILITIES OF GUM ARABIC IN BUFFERS OF I = 0·20 AND DIFFERENT pH VALUES AT 1°C. GUM CONCENTRATION = 0·66%

| pH | Mobility × $10^{-4}$ cm.$^2$ sec.$^{-1}$ volt$^{-1}$ | | |
|---|---|---|---|
| | Ascending | Descending | Difference |
| 7·79 | −0·57 | −0·54 | 0·03 |
| 6·34 | −0·57 | −0·54 | 0·03 |
| 5·08 | −0·52 | −0·49 | 0·03 |
| 4·16 | −0·49 | −0·46 | 0·03 |
| 3·05 | −0·40 | −0·36 | 0·04 |

The change of electrophoretic mobility with pH was similar to that of *cyanophylla* gum. In the pH range studied, gum arabic mobilities were always less than those of *cyanophylla* gum. The asymmetry of the electrophoretic peaks is probably caused by a slow electrophoretic component which did not separate from the major peak.

Although the difference between the electrophoretic mobilities of *cyanophylla* gum and gum arabic was small, some electrophoretic separation was obtained when a mixture of the two gums was examined by electrophoresis (Fig. 4). In this experiment, equal concentrations (0·66% each) of the gums were taken and electrophoresis was carried out at I = 0·2 and pH = 7·80. As shown in Fig. 4, the area of the slow peak, which represents the gum arabic, was much smaller than that of the fast peak, hence a great deal of the gum arabic was incorporated into the fast peak.

**Molecular weight**

Oakley[6] reported osmotic pressure molecular weights between 191,000 and 245,000 for gum arabic. Somewhat higher molecular weights (256,000 to 326,000) were obtained by Säverborn[7] by sedimentation and diffusion measurements. Presumably both were commercial samples and differed somewhat from one another and from the present sample.

The molecular weight of *cyanophylla* gum was determined by osmotic pressure measurements (Table V) in 0·2 M sodium chloride.

TABLE V

OSMOTIC PRESSURE OF *CYANOPHYLLA* GUM IN 0·2 M SODIUM CHLORIDE AT 7·5°C.

| c (Concentration grams/100 c.c.) | $\pi$ (Osmotic pressure cm. $H_2O$) | $\frac{\pi}{c}$ |
|---|---|---|
| 1·895 | 6·43 | 3·39 |
| 1·421 | 4·05 | 2·85 |
| 0·948 | 2·33 | 2·46 |
| 0·474 | 0·98 | 2·07 |

Equilibrium was established in four days. When the results of Table V are extrapolated to zero concentration, $\frac{\pi}{c}$ has a value of 1·68, corresponding to a number average molecular weight of 142,000.

In the light scattering examination of *cyanophylla* gum solutions, considerable difficulty was found in removing high molecular weight aggregates. The gum dissolved in 0·2 M NaCl was first centrifuged for an hour at 20,000 r.p.m. and then filtered through a No. 4 Pyrex sintered disc. The light scattering molecular weight found was 183,000 uncorrected for dissymmetry and a dissymmetry value $\left(\frac{I_{45}}{I_{135}}\right)$ of 1·6 was obtained. A marked anomalous increase in light scattering intensities was noticed for small angles in the forward direction, using a cylindrical scattering cell[8]. This seems to indicate that some high scattering material escaped removal during preparation of solutions. These observations throw considerable doubt on the molecular weight obtained.

In a further attempt to remove high molecular weight aggregates from the solutions, the ultrafiltration method described by Goring and Johnson[9] was used. The dissymmetry was reduced to 1·10 and a molecular weight of 132,000 was found. As, however, about 75% of the gum was held up by the filter, it was felt that a partial fractionation of the solution may have occurred during filtration. Thus, while the new value for the weight average molecular weight now agrees well with the number average molecular weight, it does not necessarily follow that this would be true for the whole sample. The question of homogeneity thus remains in doubt. This point was further examined by diffusion measurements.

In Table VI the diffusion coefficients determined in 0·2 M sodium chloride at 20°C, calculated by the maximum height, $D^{\circ}_{20}(H_m)$ and the standard deviation $D^{\circ}_{20}(\sigma^2)$ methods for *cyanophylla* gum are listed.

TABLE VI

DIFFUSION COEFFICIENTS OF *CYANOPHYLLA* GUM IN 0·2 M NaCl AT 20°C.

| Time (in seconds) | $D^{\circ}_{20}(H_m)$ | $D^{\circ}_{20}(\sigma^2)$ |
|---|---|---|
| 34,500 | 1·78<br>1·68 | 2·47<br>2·60 |
| 72,400 | 1·76<br>1·75 | 2·53<br>2·58 |
| 86,300 | 1·72<br>1·84 | 2·49<br>2·71 |
| 104,600 | 1·77<br>1·78 | 2·54<br>2·63 |
| 158,700 | 1·72<br>1·79 | 2·43<br>2·56 |

As shown in Table VI, no trend of diffusion coefficients with time was observed, but the two methods of calculation gave considerably different diffusion coefficients. This difference indicates a certain degree of heterogeneity[10, 11, 12].

To Dr. P. C. Carman I am greatly indebted for his interest and criticism at all times. This paper is published with the permission of the South African Council for Scientific and Industrial Research.

National Chemical Research Laboratory,
C.S.I.R., Pretoria.

*Received June* 11, 1954.

## REFERENCES

[1] Cook, W. H., Rose, R. C. and Colvin, J. R., *Biochem. Biophys. Acta*, 1952, **8**, 595.
[2] Colvin, J. R., Cook, W. H. and Adams, G. A., *Can. J. Chem.*, 1952, **30**, 603.
[3] Adair, G. S., *Proc. Roy. Soc.*, 1925, **A108**, 627.
[4] Brice, B. A., Halwer, M. and Speiser, R., *J. Opt. Soc. Am.*, 1950, **40**, 768.
[5] Smith, F., *J. Chem. Soc.*, 1939, 744.
[6] Oakley, H. B., *Trans. Faraday Soc.*, 1936, **32**, 1360.
[7] Säverborn, S., *Contribution to the Knowledge of the Acid Polyuronides*, Almqvist and Wiksells Boktryckeri AB, Uppsala (1945).
[8] Witnauer, L. P. and Scherr, H. J., *Rev. Sci. Instr.*, 1952, **23**, 99.
[9] Goring, D. A. I. and Johnson, P., *J. Chem. Soc.*, 1952, **33.**
[10] Gralen, N., *Inaugral Dissertation*, Uppsala, (1944).
[11] Charlwood, P. A., *J. Phys. Chem.*, 1953, **57**, 125.
[12] Herdan, G., *Nature*, 1953, **172**, 581.

# UPTAKE AND DEGRADATION OF LABELLED SYSTEMIC INSECTICIDES*

## PART II—TREATMENT OF TOBACCO AND POTATOES WITH *SYSTOX*

*by*

**L. H. STEIN and A. J. SMITH**

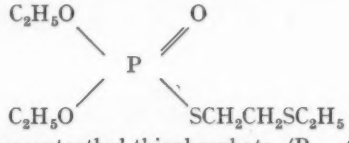
OPSOMMING

Deur gebruik van radioaktiewe *Systox* word eksperimente op tabak en aartappels uitgevoer om die hoeveelheid insekdoder wat op verskillende tye na toediening oorbly vas te stel. Nadat tabak met die insekdoder bespuit is daal die konsentrasie oor 'n tydperk van 28 dae vinnig na 'n baie lae waarde. 'n Grondtoediening vir tabak en aartappels toon dat die insekdoder, of afbreekprodukte daarvan, voortdurend en oor 'n lang tydperk opgeneem word.

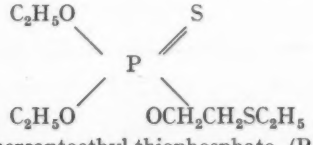
SUMMARY

Using labelled *Systox*, experiments have been carried out on tobacco and potatoes in an endeavour to evaluate the concentration of insecticide at different times after application. After tobacco has been sprayed with the insecticide, the concentration decreases rapidly and falls to a low value at 28 days. Soil applications to tobacco and potatoes, however, indicate that the insecticide or the degradation products thereof, are taken up continuously over long periods.

The systemic insecticide *Systox* was developed by Schrader[1]. In the technical product two isomeric forms are present and, due to their limited solubility, these are dispersed in water by use of an emulsifier which constitutes 50 *per cent.* of the insecticidal mixture. The active ingredients are present as 15 *per cent.* of P = O and 35 *per cent.* of P = S compounds. These forms have the following constitution:



O, O-diethyl S-ethylmercaptoethyl thiophosphate. (P = O compound.)



O, O-diethyl O-ethylmercaptoethyl thiophosphate. (P = S compound.)

Owing to its toxicity as a cholinesterase inhibitor, the use of this insecticide has been restricted to plants not intended for human comsumption. In order to ascertain how much of the insecticide or its degradation products remained at the time of harvesting or consumption, two suitable plant types were treated with *Systox*.

The level of concentration at which the insecticide is normally applied is 0·05 *per cent.* in water, and since methods of chemical or biological assay at resultant plant concentrations are virtually impossible, radiochemical techniques have been resorted to. By labelling the insecticide with $P^{32}$ (14·3 days half-life), the concentration of *Systox*, together with its degradation products in the plant, can be assessed by counting techniques.

*The first paper in this series, "The movement of radiophosphorus-labelled insecticides in groundnut plants" by Stein *et. al.* appeared in *J. Sci. Fd. Agric.*, 1952, **3**, 31.

Potato and tobacco plants were treated with labelled *Systox* and results given here relate all the radioactivity to the insecticide. It has been reported[2] that shortly after absorption by the plant, the P = O compound decomposes progressively into three toxic compounds as well as ionic products containing phosphorus. *Systox* itself and two of the toxic compounds can be separated from the other compound and the non-toxic ionic products, by suitable solvent extraction. It is thus possible to assess the concentration of the greater proportion of toxic material present relative to the other degradation products. Details of the method evolved by one of us are published elsewhere[3], but the percentage of the more toxic components relative to the total concentration is included in the results given below.

The following treatments with aqueous solutions of *Systox* were carried out: (a) spraying of tobacco; (b) soil application to tobacco; (c) soil application to potatoes. Control of sucking insects, such as aphids as well as mites and red spider, should be achieved by these treatments. In soil applications it is hoped that eelworm will also be controlled. Entomological studies were carried out separately and will be reported elsewhere.

## MATERIALS AND METHODS

**Labelled Systox.** The P = O compound, which does not isomerise, was synthesised from a $P^{32}$-containing compound by Dr. R. Muhlmann of Farbenfabriken Bayer of Elberfeld, whilst he was in South Africa.

**Unlabelled Systox.** The technical product, emulsifier and P = S compound were supplied by Messrs. Taeuber and Corssen (Pty.) Ltd.

**Quantities and activities.** In all cases the proportions of 3:7:10 were maintained for the P = O, P = S and the emulsifying compounds. Where necessary, technical *Systox* was used to dilute the radioactive mix, so that the final volumes used were all at a concentration of 0·05 *per cent.* In treatments (a), (b) and (c), 7·5, 55 and 36 gal. respectively, were used at this concentration. The activities for the three treatments were respectively: 0·5 mc. on 25th November, 1953, 4·45 mc. on 8th January, 1954 and 1·66 mc. on 29th January, 1954.

**Application and sampling.** For treatment (a), tobacco seedlings planted out on 15th October, 1953, were sprayed on 30th November, 1953. Some 280 plants were treated by means of a pressure driven hand spray-gun until they were dripping; each plant received about 100 ml. of solution. The plants were allowed to dry and ten samples were taken by stripping the leaves off five plants in rows 1 and 2, and a further five in the other two rows. Samples were similarly taken 1, 3, 7, 14 and 28 days after treatment. In order to calculate concentrations on a dry weight basis, five extra samples were collected for drying at each sampling.

On 8th January, 1954, tobacco transplanted on 23rd November, 1953, was given the second treatment, (b), by pouring 2 gal. of *Systox* solution into a frame 18 x 72 in. On the average, six plants were contained in the frame and about 156 plants were dosed. Care was taken to minimise contamination of the plants. Fifteen samples were taken 3 and 10 days later, and 17, 31 and 53 days after treatment, thirty samples were taken from fifteen plants, the top leaves being kept apart from the lower ones. At the final sampling, further picking was carried out and amalgamated samples of top leaves and also of bottom leaves were separately oven-cured and analysed.

Potatoes for treatment (c) were planted on 8th December, 1953, and the resulting plants were treated on 25th January, 1954, in the same way as the second batch of tobacco. Only 72 plants in batches of four per frame were, however, treated. Ten samples of the portion growing above ground were taken 7, 21 and 35 days after treatment, and at 56 and 92 days ten samples of tubers were lifted.

**Preparation for radiometric analysis.** After obtaining wet weights, samples were digested with nitric and sulphuric acids and excess liquid was evaporated. In treatment (a) and the first two samplings of (b), dried samples were also digested in order to determine whether any loss of activity had occurred during heating. The

preparation of samples of the toxic and non-toxic degradation products and the separation involved have been dealt with in another paper[3].

**Radiometric analysis.** After filtration and the measurement of volumes and specific gravity, solutions were counted in M12 liquid counters at concentrations such that counting rates were not subject to coincidence losses. Correction curves of percentage loss versus specific gravity were drawn up for both $P^{32}$ and $K^{40}$, and the necessary corrections were applied for density, $K^{40}$ contribution (where significant), and decay of $P^{32}$.

TABLE I

**TOBACCO : SPRAY APPLICATION**

| Days after spraying | Rain mm. | Sample | Wet weight g. | % dry weight | *Systox* p.p.m. dry weight | Total *Systox* mg. | Remarks |
|---|---|---|---|---|---|---|---|
| 0 | | W | 129·5 | 15·0±0·9 | 115 ±7·4 | 2·2 | 5 ex rows 1 and 2 |
| | Nil | W | 76·3 | | 186 ±5·4 | 2·1 | 5 ex rows 3 and 4 |
| 1 | | W | 165·8 | 13·3±0·1 | 29 ±1·7 | 0·64 | No row difference |
| | 13·6 | D | 138·5 | | 34·6 ±2·0 | 0·64 | |
| 3 | | W | 202·9 | 13·3±0·4 | 18·0 ±0·8 | 0·49 | No row difference |
| | 22·0 | D | 180·9 | | 19·7 ±1·0 | 0·47 | |
| 7 | | W | 256·6 | 13·3±0·6 | 10·6 ±1·2 | 0·36 | No row difference |
| | Nil | D | 248·5 | | 10·2 ±1·2 | 0·34 | |
| 14 | | Wp | 199·5 | 13·1±0·4 | 5·52±0·69 | 0·23 | 5 ex rows 1 and 2 |
| | | W | 326·3 | | 9·73±0·87 | 0·42 | 5 ex rows 3 and 4 soiled |
| | 33·0 | Dp | | | 6·65±0·05 | | 3 ex rows 1 and 2 |
| | | D | | | 10·3 ±1·0 | | 2 ex rows 3 and 4 soiled |
| 21 | | Wp | 277·0 | 14·0±0·4 | 6·31±0·97 | 0·31 | No row difference |
| | Nil | W | 363·6 | | | | |
| | | D | | | 5·3 ±1·3 | | |
| 28 | | Wp | 238·1 | 15·2±0·8 | 2·99±0·29 | 0·19 | No row difference |
| | | W | 422·5 | | | | Correction applied for $K^{40}$ |

W — Wet plants digested.
Wp — Wet plants digested after priming.
D — Dry plants digested.
Dp — Dry plants digested after priming.

## RESULTS

The results for treatments (a), (b) and (c) are set out in Tables I, II and III, respectively. In calculating the amount of insecticide present, degradation has been disregarded. Furthermore, although only the P = O compound was labelled, radioactivity was related to the total insecticidal constituents present, whether labelled or not. In experiments on citrus, Muhlmann[4] found that the P = S compound disappears more rapidly than does the P = O compound. Since the degree to which this occurs with tobacco and potatoes is, however, not known, this has not been allowed for, and the results will err on the high side.

Results for the *Systox* content have been based on the condition of the plant when offered for consumption, so that the tobacco results refer to dry weights and those for potatoes to wet weights. In Tables II and III, values are given for the percentages of the more toxic degradation products present relative to the total soluble activity. For the assessment of residual toxicity in the plants, these figures give a more significant idea of this level than do the other results. A discussion of this is given in Part III of this series[3].

Plants sampled immediately after spraying in rows 1 and 2 contained less *Systox* than those from rows 3 and 4. This was not the case a day later and the difference at the early stage has been attributed to the fact that the plants in rows 3 and 4 were not properly dry when sampled. No rain fell on the first day, so that the loss of activity must be due to loss by evaporation from the leaf surface, as suggested by Tietz[5]. Prior to sampling at 14 days, the lower leaves were removed from the plants in rows 1 and 2, i.e., they were primed. On analysis, it was noted that in the unprimed plant samples the lower leaves were badly soiled. Contamination from the soil could account for the higher concentrations found in these samples. Later samples were cleaner and no row discrepancies were noted.

No loss of activity on drying the samples at 96°C was found and there is consequently no loss of insecticide or at least of active material on heating. From the calculation of total *Systox* in the leaves it appears that a loss does occur with time. According to Tietz[5], this is brought about by washing out of, or evaporation from the leaves. Stems were not analysed, but it is unlikely that preferential concentration occurred there. The figures for the rainfall for the periods between sampling do not appear to have affected the results significantly.

TABLE II

**TOBACCO : SOIL APPLICATION**

| Series | Days after treatment | Leaves sampled | Wet weights g. | % dry weight | *Systox* p.p.m. dry weight | % in lower leaves w.r.t. all leaves | % more toxic cmpds. |
|---|---|---|---|---|---|---|---|
| 1 | 3 | All | 237·4 | 12·2±0·3 | 49·7± 5·0 | — | — |
| 2 | 10 | All | 387·2 | 13·1±0·4 | 150·8±15·5 | — | 42 |
| 3 | 17 | Top | 117·7 | 13·6±0·4 | 342·0±49·8 | 40·7±1·5 | 3·4 |
| | | Lower | 236·0 | 13·6±0·4 | 234·6±38·9 | | 29·0 |
| 4 | 31 | Top | 192·1 | 16·3±0·3 | 482·2±52·6 | 38·9±1·6 | 3·1 |
| | | Lower | 269·2 | 15·3±0·3 | 322·9±27·8 | | 8·0 |
| 5a | 53 | Top | 242·8 | 15·5±0·7 | 677·4±110 | 40·3±3·3 | 2·4 |
| | | Lower | 259·7 | 16·1±0·9 | 411·2±87·6 | | 0·5 |
| 5b | 53 | Cured top | — | 73·6±0·6 | 566·5±12·7 | 42·9± ? | — |
| | | Cured lower | — | 73·6±0·6 | 425·9±11·2 | | 0·4 |

Weighted means of *Systox* concentration for series 3, 4, 5a and 5b are 271±59, 389±46, 539±102 and 483±12, in p.p.m. dry weight, respectively. Considered together with the results for series 1 and 2, it appears that there is a steady increase in activity in the plants with time. For the first 17 days this is, in fact, proportional to the time, but it then tends to be taken up less rapidly. It appears that the plant draws continually on the reservoir of material in the soil, and the decrease apparent in the first trial is swamped by the steady uptake.

The second last column gives the percentage activity in the lower leaves relative to the summed activity for all the leaves. This remained remarkably constant and indicates that where there is new growth there is also the demand for nutrient. The last column gives the value for the percentage of the more toxic compounds in the water soluble portions of the plant relative to the total soluble activity and the siignificance of these figures is discussed in a further paper[3].

Comparison of series 5a and 5b indicates that there is no loss of activity on flue curing of the leaves. This is not surprising in view of the results found on drying the samples and then analysing them. In addition to the results given in Table I, this was also found for the relevant samples in series 1 and 2 of this experiment, but for simplicity these results have not been given separately in Table II.

From the levels of activity found it appears that two gallons per square yard of a 0·05 *per cent. Systox* solution is excessive, if the residual toxicity is to be kept within certain limits, and in particular when compared with the levels found when the plants were sprayed. It would be more economical and it is hoped as satisfactory for entomological control, if the concentration were halved.

TABLE III

**POTATOES: SOIL APPLICATION**

| Days after treatment | Sample | Wet weight g. | *Systox* p.p.m. wet weight | % more toxic compounds |
|---|---|---|---|---|
| 7 | Above ground | 270 | 7·34 ± 0·74 | 32·1 |
| 21 | Above ground | 355 | 42·2 ± 6·4 | 6·1 |
| 35 | Above ground | 663 | 45·6 ± 4·1 | 1·0 |
| 56 | Tubers | 564 | 122·7 ±13·7 | 0·7 |
| 92 | Tubers | 1037 | 83·6 ± 8·9 | — |

Based on wet weight, the level of apparent insecticide does not look as formidable as that in Table II, but it is nevertheless considerable. In the portion above ground there is a definite increase with time and considering the fact that at the 35 day stage there were no tubers, the level in these at the next stage indicates a persistent uptake by the plant, and that there must still have been material available in the soil, as the total taken up by the tubers is greater than was available in the plant alone. If we assume that half a gallon of solution was available per plant, then the recovery by the tubers alone accounts for about one-seventh of the applied activity. On the basis of one-third of a gallon for a tobacco plant then at the final stage the leaves contained one-twentieth of the applied activity. Potatoes therefore have a higher utilisation factor than tobacco plants.

## CONCLUSIONS

On the basis of no degradation, residual toxicities are extremely high for crops treated by application of a 0·05 *per cent. Systox* at a dosage of 2 gal. per sq. yd. On the other hand, soil application results indicate that this method should give greater and more lasting protection from insects.

The results given are for the highest possible levels, since they were based on total insecticide, although only the P = O compound was labelled and it is probable that the P = S compound disappears more rapidly. The tobacco results were based on dry weights, but when sold generally contain about 8 *per cent.* moisture. Finally, these results assume that no degradation took place, but, since degradation does in

fact take place and to a considerable extent, it would appear that final toxic levels are probably less than one-hundredth of the levels indicated.

The authors are indebted to Miss B. Papendieck and Messrs. I. J. Roth and L. E. L. Mussman for their assistance, and to the Central Tobacco Research Station for the facilities provided by them.

This paper is published by permission of the South African Council for Scientific and Industrial Research and the Division of Entomology.

Division of Entomology, Rustenburg.

National Chemical Research Laboratory,
S.A. Council for Scientific and Industrial Research, Pretoria.

*Received June*, 22, 1954.

## REFERENCES

[1] Schrader, G., *Monographien zu "Angewandte Chemie" und "Chemie-Ingenieur-Technik"* Nr. **62, 2**, *erweiterte Auflage* (1952).
[2] Heath, D. F. *et al.*, *Pest Control Report* (1953).
Hartley, G. S., *World Crops*, **4**, 397 (1952).
[3] Stein, L. H., succeeding paper.
[4] Muhlmann, R., unpublished work, private communication.
[5] Tietz, H., private communication.

# UPTAKE AND DEGRADATION OF LABELLED SYSTEMIC INSECTICIDES

## PART III—ESTIMATION OF THE MORE TOXIC DEGRADATION PRODUCTS OF *SYSTOX*

*by*

**L. H. STEIN**

OPSOMMING

'n Tentatiewe metode vir die bepaling van *Systox* en van sy meer giftige afbreekprodukte in plantmateriaal word tesame met resultate vir tabak en aartappels gegee. Die plante word gekweek in grond wat met 'n oplossing van die insekdoder benat word.

SUMMARY

A tentative method for the evaluation of *Systox* and its more toxic degradation products in plant matter is given together with results for tobacco and potatoes grown in soil dosed with a solution of this insecticide.

The systemic insecticide *Systox*[1,2] is made up of two isomeric forms, O, O-diethyl S-ethylmercaptoethyl thiophosphate and O, O-diethyl O-ethylmercaptoethyl thiophosphate, which will be referred to as the P:O and P:S compounds respectively. The P:O compound is more toxic and has a greater water solubility than the P:S compound which can, however, isomerise to the P:O form. The commercial product consists of 15 *per cent.* P:O compound, 35 *per cent.* P:S compound and 50 *per cent.* emulsifier and is normally applied as a 0·05 *per cent.* solution in water.

Due to its toxicity to mamals the use of this insecticide has been restricted to non-edible crops as little is known of the residual amounts remaining after treatment and subsequent growth. At the level of concentration which occurs in the plant, chemical methods of analyses are not suitable, and for this work radiometric methods of analysis have been used.

Analysis on this basis has hitherto related the radioactivity found to the amount of insecticide by calculation from the specific activity of the originally applied solution. It was felt, however, that *Systox* undergoes degradation in the plant and as a result the toxicity would probably decrease with time and would certainly be lower than the level indicated by the radioactive content. Where this level is within the probable tolerance a greater margin of safety obviously exists, but if the allowable limit is apparently exceeded it is desirable to know how much of the radioactivity is in fact due to toxic materials and what the concentration of these materials is.

## MATERIALS AND METHOD

The labelled P:O compound was of the second batch referred to in Paper II[3].

In selecting a solvent which would preferentially extract *Systox* from an aqueous solution, use was made of the fact that the P:O and P:S compounds undergo acid and alkaline hydrolysis. Extraction was found to be complete with volume of chloroform equal to the volume of aqueous solution.

Where the material to be extracted was contained in plant matter, this was first cut up with a sharp knife and the shredded material macerated with a Waring

Blendor in sufficient water. Filtration of the macerate was improved with Hyflo Supercel filter-aid and residual plant matter was washed twice with water. This did not free it of all the aqueous extract but further washing was avoided as this would greatly have increased the volumes and the resulting solutions would have been too low for accurate counting. Digestion of the residual plant matter allowed an assessment to be made of the activity in this portion and together with that in the aqueous extract gave the total activity in the plant. The last column of figures in Tables I and II is for the efficiency of the extraction with water relative to the total activity in the plant. The extraction was by no means complete and this may be ascribed to inefficiency of washing, incomplete maceration, and to absorption and adsorption or actual insolubility of products in the plant matter. It has been assumed that the aqueous extract is representative of the activity content in the plant and that the chloroform values are thus not affected by the different efficiencies of aqueous extractions.

In some cases the aqueous extracts were not clear but centrifuging led to sedimentation of the suspended solids. The activity carried down with these solids was not chloroform-extractable and as the concentration of chloroform-extractable activity relative to total soluble activity was required it was necessary to clarify all the aqueous extracts before chloroform extraction. For purposes of obtaining the total activity the sedimented activity was counted in with the solid which had remained on the filter.

Extraction of normal solutions with chloroform was straight-forward but plant solutions had to be recentrifuged after shaking with chloroform as very thick emulsions were formed in the organic layer. Centrifuging at 3,000 rpm. for 10 minutes was usually sufficient to separate the two layers leaving a pad of insoluble material between them. It was found that this pad was largely composed of the same material as was precipitated on saturating the plant solution to 80 *per cent.* with ammonium sulphate. The activity accompanying this precipitate was roughly equivalent to that extracted from the original solution with chloroform. Treatment of the precipitate with this solvent resulted in a transfer of activity from the solid into the organic layer. As was also shown experimentally, breaking of the emulsion thus had no effect on the activity extracted by the chloroform.

All solutions were counted in an M 12 liquid counter and corrections for the density effect of the organic solvent and for decay of the $P^{32}$ were applied. Comparison of the different solutions was therefore possible and all activity could be accounted for.

## EXPERIMENTAL

In establishing the method a 0·05 *per cent.* solution of *Systox* was diluted twenty times with water. On extraction with chloroform all but 4·5 *per cent.* of the activity was chloroform soluble. On acidification (0·01M) of the aqueous solution no immediate change of non-extractable activity was noted. Even after 18 h. very little change had occurred. On addition of sodium hydroxide (0·01M), however, the non-extractable portion increased to 98 *per cent.* at 18 h. In order to check whether non-extractability was due to the formation of a sodium salt and not to degradation, the acidified solution was treated with sodium bicarbonate and the alkaline solution with acid. Both treated solutions were immediately chloroform extracted but no changes were observed. It was thus apparent that *Systox* was readily extracted but that the hydrolysis products were not.

In order to obtain definite evidence of the reliability of the method the following three experiments were carried out. A solution containing 0·05 *per cent. Systox* was extracted with chloroform and it was found to contain 85 *per cent.* extractable material. The roots of a tobacco plant were immersed in this solution and 24 and

48 h. later some leaves were removed for treatment and evaluation of the chloroform soluble fraction. The percentage found in both cases was 77·5 indicating a slight loss. The remaining nutrient solution was also examined both with respect to concentration of activity and extractable material. These had not altered in spite of the fact that some 40 *per cent.* of the solution had been absorbed by the plant. It would thus appear that the plant made no distinction between the water, the *Systox* or its degradation products.

A second plant was sprayed with a solution containing 76 *per cent.* chloroform extractable activity. On analysis of leaves 24 h. later this extractable activity was found to be 67 *per cent.* Lastly, to a sample of shredded plant material a solution containing 59 *per cent.* chloroform extractable material was added. This mixture was macerated, left for two h., filtered and the solution obtained was extracted with chloroform. This extract contained 55 *per cent.* of the activity of the plant solution.

In all three cases therefore, though a slight decrease in chloroform extractable material occurred, the amount extracted was substantially the same and indicates that the method is at least 90 *per cent.* efficient.

Towards the latter stages of the main experiments when chloroform activities were low, it was felt that these might be due to slight solubility of normally non-extractable matter. Plant solutions were accordingly treated with alkali and left overnight. On extraction with chloroform it was found that there was no significant difference from background in the chloroform activity, which indicates that the low values obtained were true values of the extraction.

Before presentation of results obtained on field trials with tobacco and potatoes it is desirable to discuss the above method in the light of a report which came to the author's notice after the major portion of the work had been completed. This insecticide has been investigated at Pest Control Ltd. in England, and it is stated in their report[4] that *Systox* is degraded very rapidly after entry into the plant, as a result of which, four groups of degradation products were found for the P:O compound. These are referred to as D1, D2, D3 and ionic. The members of the last group will, by their very nature, be non-toxic. Separation by successive solvent partition yielded the pure P:O, D1 and D2 compounds in solution. Difficulty was experienced in obtaining the D3 compound pure as the solvent used also dissolves various plant constituents. The toxicity to rats and mice of the various fractions was tested and it appears that the toxicities are in the order P:O>D1>D2>D3.

According to the above report all but the D3 and ionic compounds are extracted with chloroform so that the method proposed and used by the author gives the concentration of the more toxic degradation products. It is regretted that the above report was not available earlier so that an attempt could have been made to assess the concentration of the final degradation product D3 in the aqueous phase after chloroform extraction.

## RESULTS

The results obtained for tobacco and potatoes grown in soil which received a dosage of 2 gal. per sq. yd. of a 0·05 *per cent. Systox* solution, are tabulated below. Those referred to as p.p.m. *Systox* were calculated on the assumption that no degradation occurred. It will be seen that in the case of tobacco this is based on dry weights and in that of potatoes on wet weights. This was done since the two crops are presented for consumption in these states. Percentages for the more toxic compounds relative to the total soluble activity are listed, and in the last column the percentage of activity in the plant solution relative to total plant activity is given. (This has been discussed above).

TABLE I

TOBACCO

| Series | Days after treatment | Sample | *Systox* p.p.m. dry wt. | % in $CHCl_3$ extract | % in $H_2O$ solution |
|---|---|---|---|---|---|
| 2 | 10 | All | 150·8± 15·5 | 42 ±15 | 78 |
| 3 | 17 | Top half | 342·0± 49·8 | 3·4± 1·3 | 60 |
| | | Lower half | 234·6± 38·9 | 29·0± 3·0 | |
| 4 | 31 | Top half | 482·2± 52·6 | 3·1± 1·4 | 62 |
| | | Lower half | 322·9± 27·8 | 8·0± 1·3 | |
| 5a | 53 | Top half | 677·4±109 | 2·4± ·25 | 40 |
| | | Lower half | 411·2± 88 | ·5± ·3 | |
| 5b | 53 | Top half | 566·5± 13 | — | — |
| | | Lower half | 425·9± 11 | ·4± ·2 | 55 |

As may be seen the top leaves were analysed independently of the lower leaves. It is interesting to note that whereas the total activity in the tops was higher the toxic concentration was lower than in the bottom leaves. This might be ascribed to increased metabolic degradation in the faster growing portions with the older leaves acting as a reservoir of toxic material.

A definite decrease in the chloroform extractable material occurs. The errors involved are large but apart from the first result which was obtained during the development stage they can be taken as significant. From these results it can be seen that whereas the highest possible toxic content is in the region of 500 p.p.m. the content of the more toxic materials is nearer 5 p.p.m., which is of great importance in a discussion on the feasibility of using this insecticide on this crop.

Series 5a and 5b were harvested at the same time but whereas 5a was treated in the normal way 5b consisted of amalgamated samples of top leaves and of lower leaves which were then flue cured before analysis. This curing process had no effect on either the total content or on the extractable activity.

TABLE II

POTATOES

| Series | Days after treatment | Sample | *Systox* p.p.m. wet wt. | % in $CHCl_3$ extract | % in $H_2O$ solution |
|---|---|---|---|---|---|
| 1 | 7 | Plants above ground | 7·34± 0·74 | 32·1 ±1·7 | 62 |
| 2 | 21 | ,, | 42·2 ± 6·4 | 6·1 ±0·5 | 68 |
| 3 | 35 | ,, | 45·6 ± 4·1 | ·99±0·24 | 53 |
| 4 | 56 | Tubers | 122·7 ±13·7 | ·71±0·27 | 66 |
| 5 | 92 | ,, | 83·6 ± 8·9 | — | — |

This experiment was carried out over a considerably longer period than the previous one and it is unfortunate that the final result with regard to the more toxic products could not be measured. The activity, however, after the $P^{32}$ had decreased through more than six half-lives, was too low to allow for a further decrease

of about 99 *per cent.* on extraction. A steady decrease in concentration of the extractable activity is apparent and it is very probable that the missing figure would be in the region of 0·5 *per cent.* This means that whether the potatoes were consumed at the early or the mature stage the concentration of the more toxic compounds would not have exceeded 1·2 p.p.m. The fact that these results are based on wet weights must be emphasised; on a dry weight basis the figure would have been in the region of 5 to 8 p.p.m.

## CONCLUSIONS

From the experimental work it appears essential that some form of extraction technique must be applied for the evaluation of residual toxic material. The method suggested here is only partially successful and for a full assessment to be made the D3 compound must also be separated. In the report mentioned earlier it is stated, however, that: "The hazard from D3 seems known to be only about 1/7th of that from D1 and D2. With more knowledge of its properties it might be possible to ignore it safely under some circumstances." Whether the circumstances referred to apply to this particular case is not known but the author feels that the hazard from the D3 material is not a large one.

## ACKNOWLEDGEMENTS

It is with gratitude that I extend my thanks to Miss B. Papendieck and Mr. I. J. Roth for their assistance.

Permission for publication has been kindly granted by the South African Council for Scientific and Industrial Research.

National Chemical Research Laboratory,
South African Council for Scientific and Industrial Research,
Pretoria. *Received June*, 22, 1954.

## REFERENCES

[1] Schrader, G., Monographien zu „Angewandte Chemie" und „Chemie-Ingenieurtechnik" Nr. **62** 2, erweiterte Auflage, 1952.
[2] Ripper, W. E., Paper read at IIIrd Int. Con. of Crop Protection, 1953.
[3] Stein, L. H., and Smith, A. J., preceding paper.
[4] Heath, D. F., *et al*, Pest Control Report, 1953.
Hartley, G. S., *World Crops* (1952) **4**, 397.

# FERTILISER STUDIES WITH TOBACCO PLANTS USING RADIOPHOSPHORUS-LABELLED SUPERPHOSPHATE

## PART IV

*by*

**L. H. STEIN and H. L. PEARSE**

OPSOMMING

Verdere eksperimente word beskryf om die gebruik van toegediende fosfaat op tabak te meet. Drie verskillende hoeveelhede van 'n 4N.12P.6K kunsmis bevatende radio-aktiewe superfosfaat word aan verskeie grondsoorte toegedien, waarvan bekend is dat hulle met betrekking tot beskikbare fosfaat- inhoud verskil. In alle gevalle het toedienings van kunsmis ten gevolge dat die opname van kunsfosfaat vermeerder, terwyl die persentasie herwinning van toegediende fosfaat min of meer konstant bly. Hierdie herwinning verskil egter van grondsoort tot grondsoort.

SUMMARY

Further experiments on the utilisation of added phosphate to tobacco are described. A 4N.12P.6K fertiliser mixture containing labelled superphosphate has been applied at three levels to a number of soils known to differ in their available phosphate content. In all cases addition of fertiliser results in increased uptake of fertiliser phosphate with the percentage recovery of applied phosphate remaining more or less constant. This recovery, however, varies from soil to soil.

### INTRODUCTION

In continuation of the work previously reported[1] on the utilisation of phosphates by tobacco, further experiments are described which were designed to study the uptake of fertiliser phosphate applied to a number of soils known to differ fairly widely in their available soil phosphate content.

### MATERIALS

The superphosphate used was prepared at the Research Department of Messrs. African Explosives and Chemical Industries, Ltd., at Modderfontein. A single batch was used which contained 20·8 *per cent.* total and 16·8 *per cent.* water-soluble $P_2O_5$. The specific activity was 488 $\mu$rd per mg. $P_2O_5$ on 20th November, 1953.

Nitrogen and potassium were applied independently of the superphosphate as ammonium sulphate and muriate of potash.

The plants used were of the Orinoco type, Yellow Mammoth x Vamorr variety, and were grown at the Central Tobacco Research Station prior to transplantation. Transplantation to the various experimental sites was carried out when the plants were 10 weeks old. The sites were chosen as follows: a soil derived from diabase and a black noritic turf on the Research Station, two sandy loams at Rustenburg, and a red loam and a red clay loam near Brits. Soil analysis showed that these soils were of varying phosphate content.

## EXPERIMENTAL

On each plot four rows of twenty-eight plants were treated. The control row received only nitrogen and potassium: 800 lb. per morgen of a 4N.6K mixture. The other rows were treated with a 4N.12P.6K mixture in amounts of 800, 1,200 and 1,600 lb. per morgen respectively. These different treatments will be referred to as $P_0$, $P_1$, $P_2$, $P_3$. The different experimental sites served as "blocks" for the purpose of replication.

Samples were taken approximately 85 days after planting by cutting off plants one inch above ground, and for each sample four plants were amalgamated. Five samples were taken per row, except in the $P_2$ and $P_3$ treatments on site 3, where plants had died and there were only sufficient for three samples. Samples were dried and milled and sub-samples taken for analyses.

## CHEMICAL AND RADIOMETRIC ANALYSIS

Where required, plants were wet-ashed with a mixture of nitric and sulphuric acids. Total phosphate was determined by the method previously described[1,2] Potassium was determined by the method reported by Wittig and Raff[3], whereby the element is precipitated with the sodium salt of boron tetraphenyl.

Radiometric determination of assimilated fertiliser phosphate using solutions was not satisfactory as the level of activity was too low, and the hollow briquet method[4] was used. Thirty grams of milled plant material containing about 8 *per cent.* moisture was pressed to form hollow briquets. Three of these were slipped over a B12 counter (20th Century Electronics, Ltd.) for estimation of activity. In this way counting rates were improved approximately tenfold. A conversion factor was established for relating the briquet counts to those for digested samples.

Because of the relatively low counting rates, the contribution to counting rate by $K^{40}$ was high. Corrections for this were made by determination of potassium and the counting of briquets from the controls. A relation between counting rate per 90 g. and the percentage of potassium was thus obtained, and from a knowledge of the potassium content in the other samples the $K^{40}$ contribution could be allowed for. The resultant counting rates were then corrected for decay of $P^{32}$ and results were calculated as mg. $P_2O_5$ per g. dry weight.

## RESULTS

The mean dry weight yield per four plants at each experimental site, together with the meaned results of the chemical and radiometric analyses for phosphate in the plant material, are set out in Table I. The percentage of fertiliser phosphate in the plant for each soil and for each treatment is shown. In the last column the "A value" is given. This has been obtained from the formula given by Fried and Dean[5], namely:

$$A = \frac{B\,(1 - y)}{y}$$

where $A$ = lb. per morgen available $P_2O_5$ in soil,

$B$ = lb. per morgen $P_2O_5$ applied, and

$y$ = the fraction of phosphate taken up which originates in the fertiliser.

The figures for original phosphate content of the various soils in the first column are for acid soluble and easily absorbed phosphate determined by the Bray[6] method.

The most important findings indicated from Table I are as follows:

TABLE I

**Plant Yield and $P_2O_5$ Concentration**

| Site, soil and $P_2O_5$ content | Treatment | Dry wt. g. | Total $P_2O_5$ mg. per g. dry wt. | Fert. $P_2O_5$ mg. per g. dry wt. | Fert. $P_2O_5$ / Tot. $P_2O_5$ % | "A value" lb. $P_2O_5$ per morgen |
|---|---|---|---|---|---|---|
| 1 | $P_0$ | 108·5 | 2·454 | — | — | — |
| Diabase-derived | $P_1$ | 559·3 | 2·213 | 1·317 | 59·5 | 65 |
| clay loam | $P_2$ | 662·7 | 2·326 | 1·434 | 61·6 | 89 |
| 10 p.p.m. | $P_3$ | 843·0 | 2·447 | 1·570 | 64·1 | 108 |
| 2 | $P_0$ | 1,107·1 | 3·440 | — | — | — |
| Norite | $P_1$ | 1,325·5 | 3·319 | 0·678 | 19·7 | 391 |
| turf | $P_2$ | 1,328·2 | 3·702 | 0·983 | 26·5 | 399 |
| 36 p.p.m. | $P_3$ | 1,255·8 | 3·628 | 1·089 | 30·0 | 448 |
| 3 | $P_0$ | 298·9 | 2·943 | — | — | — |
| Sandy | $P_1$ | 449·6 | 3·112 | 0·606 | 19·4 | 398 |
| loam | $P_2$ | 411·1 | 3·366 | 1·181 | 35·0 | 267 |
| 30 p.p.m. | $P_3$ | 478·7 | 3·640 | 1·401 | 38·2 | 311 |
| 4 | $P_0$ | 751·7 | 4·798 | — | — | — |
| Sandy | $P_1$ | 759·3 | 4·542 | 0·438 | 9·6 | 904 |
| loam | $P_2$ | 838·9 | 4·496 | 0·488 | 10·8 | 1,189 |
| 36 p.p.m. | $P_3$ | 803·3 | 3·667 | 0·734 | 20·0 | 768 |
| 5 | $P_0$ | 391·3 | 3·645 | — | — | — |
| Red magnetite | $P_1$ | 834·9 | 3·018 | 0·373 | 12·3 | 684 |
| laom | $P_2$ | 877·5 | 3·037 | 0·433 | 14·2 | 870 |
| 38 p.p.m. | $P_3$ | 818·3 | 2·964 | 0·509 | 17·2 | 924 |
| 6 | $P_0$ | 640·7 | 2·487 | — | — | — |
| Red magnetite | $P_1$ | 611·0 | 2·626 | 0·125 | 3·9 | 2,365 |
| clay loam | $P_2$ | 698·8 | 2·818 | 0·171 | 6·0 | 2,254 |
| 70 p.p.m. | $P_3$ | 802·3 | 2·870 | 0·229 | 7·9 | 2,238 |
| L.S.D. (P=0·05) | | 147·0 | 0·773 | 0·132 | 4·4 | 189 |

**Site 1**

Clay loam derived from diabase, available phosphate 10 p.p.m. $P_2O_5$.

Growth, as measured by dry weight, was greatly increased by the application of fertiliser and with the heavier applications further smaller but significant increases in growth occurred. In spite of these very significant growth differences, the total phosphate present per g. of dry matter was not significantly different for the various treatments. The general level is, in fact, low. From the fertiliser phosphate figures, it is clear that there was a very considerable uptake of fertiliser phosphate and the percentage of fertiliser phosphate in the plant was high, varying from 59·5 *per cent.* for $P_1$ to 64·1 *per cent.* for $P_3$. The "A value" varied from 65 to 108, which is comparatively low and is indicative of the low level of available phosphate in the soil.

**Site 2**

Noritic turf soil, available phosphate 36 p.p.m. $P_2O_5$.

In this soil growth was not significantly improved by the application of phosphate, although the control plants, without applied phosphate, were slightly later in coming into bloom. The general level of total phosphate present in the plant material was not significantly different for the different treatments, although tending to be slightly higher with the heaviest phosphate application. Fertiliser phosphate absorption increased with increasing application, as well as the percentage fertiliser phosphate in the plants which varied from 19·7 to 30·0 *per cent.* The "A value" varied from 391 to 448, indicating a fair level of available phosphate in the soil.

**Site 3**

Light sandy loam, available phosphate 30 p.p.m. $P_2O_5$.

On this soil growth was poor and was to some extent affected by eelworm infestation. The growth of the phosphate treated plants was slightly better than the untreated, but between levels of application no significant difference was apparent. Phosphate uptake and "A values" were very similar to those of the previously described site 2.

**Site 4**

Sandy loam, available phosphate 36 p.p.m. $P_2O_5$.

Growth was very good, but was not significantly affected by phosphate level. Total phosphate concentration in the plants was high, but differences between treatments were not significant. Slightly more fertiliser phosphate was taken up with the higher phosphate application. The percentage of fertiliser phosphate in the plants varied from 9·6 to 20·0 *per cent.* These comparatively low figures, therefore, gave high "A values" of from 768 to 1,189. Available soil phosphate is, therefore, at quite a high level and it is known that considerable applications of superphosphate had been made to this soil during previous years.

**Site 5**

Red magnetite loam, available phosphate 38 p.p.m. $P_2O_5$.

On this soil a very definite improvement in growth resulted from the application of phosphate, although differences between the various levels of application were not significant. More fertiliser phosphate was taken up with increasing application and the percentage fertiliser phosphate in the plants varied from 12·3 to 17·2 *per cent.* The "A value" varied from 684 to 924 and the available soil phosphate is, therefore, apparently at a fairly high level.

**Site 6**

Red magnetite clay loam, available phosphate 70 p.p.m. $P_2O_5$.

There was no definite influence on growth from the application of phosphate, although this may have been at least in part due to infestation by eelworm. The total phosphate content of the plants was rather low and the percentage fertiliser phosphate was extremely low, varying from 3·9 to 7·9 *per cent.* The "A value" was consequently very high and this is in accord with the high value for available phosphate. The term "available" is, however, a misnomer in this case.

In Table II the total phosphate and the fertiliser phosphate uptake per four plants at harvest are shown, together with the difference between these amounts, which gives a figure for soil phosphate absorption for each treatment. In the last column the percentage recovery of the applied fertiliser phosphate is also given.

TABLE II

**Plant $P_2O_5$ Content as Derived from Fertiliser and Soil Phosphate**

| Site | Treatment | Total Fert. $P_2O_5$ g. | Total $P_2O_5$ g. | Soil $P_2O_5$ g. | Recovery of $P_2O_5$ % |
|---|---|---|---|---|---|
| 1 | $P_0$ | — | 0·266 | 0·266 | — |
| | $P_1$ | 0·737 | 1·238 | 0·501 | 9·0 |
| | $P_2$ | 0·950 | 1·541 | 0·591 | 7·7 |
| | $P_3$ | 1·326 | 2·063 | 0·637 | 8·1 |
| 2 | $P_0$ | — | 3·808 | 3·808 | — |
| | $P_1$ | 0·899 | 4·399 | 3·500 | 10·9 |
| | $P_2$ | 1·306 | 4·917 | 3·611 | 10·6 |
| | $P_3$ | 1·368 | 4·556 | 3·188 | 8·3 |
| 3 | $P_0$ | — | 0·880 | 0·880 | — |
| | $P_1$ | 0·272 | 1·399 | 1·127 | 3·3 |
| | $P_2$ | 0·486 | 1·384 | 0·898 | 3·9 |
| | $P_3$ | 0·671 | 1·742 | 1·071 | 4·1 |
| 4 | $P_0$ | — | 3·607 | 3·607 | — |
| | $P_1$ | 0·334 | 3·449 | 3·115 | 4·1 |
| | $P_2$ | 0·409 | 3·772 | 3·363 | 3·3 |
| | $P_3$ | 0·590 | 2·946 | 2·356 | 3·6 |
| 5 | $P_0$ | — | 1·426 | 1·426 | — |
| | $P_1$ | 0·311 | 2·520 | 2·209 | 3·8 |
| | $P_2$ | 0·380 | 2·665 | 2·285 | 3·1 |
| | $P_3$ | 0·417 | 2·425 | 2·008 | 2·5 |
| 6 | $P_0$ | — | 1·593 | 1·593 | — |
| | $P_1$ | 0·076 | 1·604 | 1·528 | 0·9 |
| | $P_2$ | 0·119 | 1·969 | 1·850 | 1·0 |
| | $P_3$ | 0·184 | 2·303 | 2·119 | 1·1 |
| L.S.D. (P=0·05) | | 0·140 | 0·490 | 0·523 | 0·9 |

## DISCUSSION OF RESULTS

On all soils, increasing the dressing of the fertiliser mixture resulted in increased uptake of fertiliser phosphate, with the percentage recovery of applied phosphate remaining more or less constant. Percentage recovery, however, varied very considerably between the different sites. Recovery was better on site 2, noritic turf soil, with available soil phosphate at 36 p.p.m. $P_2O_5$, than at site 1, diabase-derived soil, with available soil phosphate at 10 p.p.m. $P_2O_5$. The lowest percentage recovery occurred on the red magnetite soil, site 6, with the soil phosphate at the high level of 70 p.p.m. $P_2O_5$. The other soils gave intermediate values.

In general it appears clear the the lower the level of available soil phosphorus, the higher the percentage of the phosphorus in the tobacco coming from the applied fertiliser. The relationship between the percentage recovery of applied phosphate and the available soil phosphate is, however, not quite clear. Thus, for example, the recovery is better on site 2, noritic turf soil, than on the extremely phosphate deficient site 1, diabase-derived soil. It seems probable, therefore, that several factors affect the recovery of applied phosphate. Such factors may well be: (a) fixation of phosphate by chemical or microbiological means in the soil, (b) variation in the type and manner of root growth due to factors such as soil structure and texture, and (c) variation in root growth and phosphate absorption due to interactions with other plant nutrients.

Future work will be directed toward the elucidation of the relative importance of these different factors on phosphate absorption.

The authors are indebted to Miss B. Papendieck, Miss S. Muhl and Mr. I. J. Roth for their valuable assistance.

This paper is published by permission of the South African Council for Scientific and Industrial Research and the Central Tobacco Research Station.

National Chemical Research Laboratory,
S.A. Council for Scientific and Industrial Research, Pretoria.

Central Tobacco Research Station, Rustenburg.

*Received June* 22, 1954.

## REFERENCES

[1] Marais, J. S. C. and Stein, L. H., *Agric. Ed. and Res. Science Series* No. 9, Bulletin No. 213 (1952).
Stein, L. H. and Marais, J. S. C., *S.A. Ind. Chem.*, 1952, **6**, 147.
Pearse, H. L. and Stein, L. H., *This Journal*, 1953, **6**, 25.
[2] Piper, C. S., "Soil and plant analysis", Interscience Publishers Inc., N.Y., 1947, 291.
[3] Wittig, G. and Raff, P., *Liebigs Ann.*, 1950, **573**, 195.
[4] Kristjanson, A. M., Dion, H. G. and Spinks, J. W. T., *Can. J. of Technol.*, 1951, **29**, 496.
[5] Fried, M. and Dean, L. A., *Soil Science*, 1952, **73**, 263.
[6] Bray, R. H. and Kurtz. L. T., *Soil Science*, 1945, **59**, 39.

# BITTER PRINCIPLES OF THE CUCURBITACEAE

## PART II*—PAPER CHROMATOGRAPHY OF BITTER PRINCIPLES AND SOME APPLICATIONS IN HORTICULTURAL RESEARCH.

*by*

**P. R. ENSLIN, T. G. JOUBERT and S. REHM.**

OPSOMMING

Die aanwesigheid van bitterheid in gekweekte lede van die komkommer-familie in Suid-Afrika word bespreek, asook die oorsprong en oorerwing van hierdie eienskap. 'n Tegniek vir die skeiding van bitterstowwe op formamied-ge-impregneerde papier word beskrywe en die moontlike toepassings van hierdie metode in die studie van die betrokke biologiese probleme word opgesom.

SUMMARY

The occurrence of bitterness in cucurbits cultivated in South Africa and the origin and heredity of this character are discussed. A technique for the separation of bitter principles on formamide-impregnated paper is described. Possible applications of this method to the study of the biological problems involved are enumerated.

## INTRODUCTION

The occurrence of bitter and poisonous forms of cultivated species of the family Cucurbitaceae in South Africa, which has been reported several times[1, 2, 3, 4], can be a grave danger if such vegetables are cooked and seasoned. The bitter substances formed in this family are so toxic that the consumption of relatively small quantities in food can cause serious illness or even death.

Bitterness is very widely found in wild species of the Cucurbitaceae. Several crystalline chemically related substances have been isolated[5], viz. cucurbitacin A, $C_{28}H_{40}O_8$, from *Cucumis myriocarpus* Naud. and *C. leptodermis* Schweickerdt; cucurbitacin B, $C_{28}H_{40}O_7$, from *Cucumis africanus* L.f., *C. metuliferus* E. Mey and *Lagenaria leucantha* (L.) Rusby; cucurbitacin C, $C_{28}H_{42}O_7$, from *Cucumis sativus* L. and cucurbitacin D from *Cucurbita pepo* L. var. *ovifera* Alef. A research programme on the following lines is being conducted at the Horticultural Research Station, Pretoria, in collaboration with the Council for Scientific and Industrial Research, *viz.*: survey of occurrence of bitterness in all cultivated Cucurbitaceae; identification of principles causing bitterness in the different species and varieties; mode of inheritance of bitterness; origin of genes for bitterness; biochemistry and physiology of bitter compounds and their possible precursors.

In this communication the preliminary results from the above horticultural research programme are discussed and paper chromatographic methods for the identification and approximate estimation of bitter constituents are described.

## OCCURRENCE OF BITTERNESS IN CULTIVATED CUCURBITACEAE IN SOUTH AFRICA.

Bitter specimens have been found of the following cucurbits, cultivated for their fruits in South Africa:

*Cucurbita pepo* L., squash and vegetable marrow. Bitter fruits have been reported from a number of varieties of this species. They seem to occur most frequently in the variety Little Gem, whereas bitterness is only occasionally found in the varieties Golden Custard and Long White Bush (erroneously referred to as "species of *Cucumis*" by Steyn[2, 3]). To the same species belong the ornamental gourds which have only decorative value and are generally bitter.

---

* Part I, *J. Sci. Fd. Agric.*, 1954, **5**, 410.

*Citrullus vulgaris* Schrad., water-melon. The wild *C. vulgaris* occurs in several forms in Southern Africa, some of which are bitter. Bitterness in the cultivated red-fleshed varieties is apparently of rare occurrence.

*Cucumis sativus* L., cucumber. The bitterness which can develop in certain varieties, especially in some of the European hot-house cucumbers, is rarely found under South African conditions.

*Cucumis metuliferus* E. Mey., jelly melon. This plant is indigenous to South Africa and occurs in bitter and non-bitter forms in the wild state. The non-bitter form is sometimes cultivated.

*Lagenaria leucantha* (L.) Rusby, maranca, calabash. Bitter marancas occur rather frequently. Steyn's reference[1] to this plant as "*Cucumis* spec." is incorrect.

*Luffa acutangula* Roxb., luffa. The flesh of this rarely cultivated plant is non-bitter, but the seeds contain a bitter principle.

## HEREDITY AND PHYSIOLOGY OF BITTERNESS

Very little work has hitherto been done on the inheritance of bitterness in the family Cucurbitaceae. Pathak and Singh[6] and Barham[7] studied the fruit taste character, or bitterness, of the calabash and cucumber respectively. It was concluded that the bitter principle is controlled by a single dominant gene. The gene for bitterness is not linked with any of the other plant characters studied.

In our genetical investigations we divide water-melons and Little Gem squashes into three classes according to taste, *viz.* slightly bitter, bitter and very bitter. This may indicate that multiple factors are involved. It would be highly desirable to base the genetical analysis on an exact quantitative determination. In the case of bitter *Cucumis sativus*, we suspect that the heterozygote contains less cucurbitacin than the homozygous bitter parent. This, however, must also be confirmed by a quantitative estimation.

The level of bitterness in greenhouse cucumbers may be influenced by physiological factors. Vogel[8, 9] showed that here bitterness is favoured by strong illumination, dry air, sudden rise in day temperature, low night temperatures and a high level of nutrition. Internal factors may also contribute to bitterness, the highest incidence of bitter fruits being always found in old plants.

Most of the newly developed cucumber varieties never show any degree of bitterness, even when cultivated under adverse climatic conditions. It is therefore reasonable to assume that these varieties are without the gene or genes for bitterness.

The vegetative parts of cucurbits bearing bitter fruits are generally not or only slightly bitter. Young fruits of *Cucumis metuliferus* and *C. myriocarpus* have a much less intense bitter taste than ripe fruits on the same plants. Very young fruits of bitter water-melon are, however, already very bitter, and bitter plants of this species can be identified at an early stage from the bitter taste of the young vines. On several occasions we found that fruits of cucurbits, which were bitter when collected, had lost their bitter taste completely when examined a few days later. This happened with nearly ripe fruits of cucumber (variety Early Fortune), young fruits of bitter *Cucumis metuliferus* and unripe bitter marancas. It appears that in certain varieties, at certain stages of development, the bitter principle is rather quickly destroyed. This phenomenon might be of practical importance and will be further investigated.

A genetical study of bitter principles necessitates the isolation of pure lines. By means of paper chromatography, single plants can now be selected on a chemical basis.

## ORIGIN OF BITTERNESS IN CULTIVATED FORMS

The ancestors of most of the cultivated cucurbits were probably bitter. In some cases, however, bitter and non-bitter forms of the wild species are known (e.g., *Citrullus vulgaris*, several *Cucumis* species).

Possible explanations for the origin of bitterness in cultivated forms are:

(a) intergeneric of interspecies crosses with bitter forms;
(b) crosses with bitter forms of the same species;
(c) back mutation of a single gene to give rise to the ancestral bitter taste (atavism).

Steyn[3], [4] advanced the theory that bitter vegetable marrows and bitter water-melons originated from a cross between the cultivated cucurbits and wild bitter species of *Cucumis*. This possibility can be excluded since all intergeneric crosses attempted in this family have been unsuccessful. Even interspecies crosses are usually impossible (*e.g.*, between *Cucumis melo* and *Cucumis sativus*, two closely related species). In the few cases of successful experimental interspecies crosses (*e.g.*, between *Cucurbita pepo*, *C. maxima* and *C. moschata*) the hybrids are usually sterile [10, 11].

Some cases investigated in the Little Gem squash and calabash suggest a cross between sweet cultivated and wild bitter types of the same species. After self-pollination, the plants segregated for bitterness, fruit shape and colour. It therefore appears as if bitterness in the Little Gem squash is the result of a cross between normal plants and a closely related bitter type, presumably ornamental gourds. Bitter and sweet calabashes cross readily when grown in the same field.

The third possibility to be considered is a back mutation of a single gene to give rise to the ancestral bitter taste.

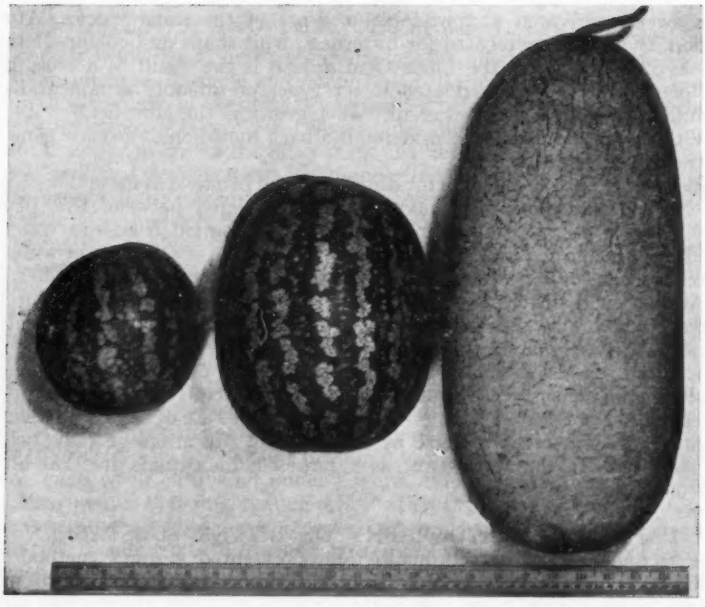
A single bitter water-melon plant was discovered in a field of more than a hundred acres. No morphological differences could be detected between this plant and ordinary sweet types (see Fig. 1 in Steyn[4]). Seeds planted from this water-melon produced both bitter and sweet plants, showing that the original bitter water-melon was heterozygous for bitterness. The characters of skin colour and flesh colour in South African wild bitter water-melons are dominant over those shown by the cultivated variety Hawkesbury (see Fig. 1). Further, when the bitter cultivated water-melon was self-pollinated, no segregation for fruit shape, skin colour or flesh colour occurred. It is thus most unlikely that the bitter cultivated water-melon was the result of a cross between a wild bitter and a sweet cultivated plant. Apparently the bitterness in the cultivated water-melon (and in one case of the Little Gem squash investigated) was therefore caused by a mutation. Mutations usually behave as simple recessives. In the above two cases, however, indications are that bitterness behaves as a dominant factor.

## PAPER CHROMATOGRAPHY OF BITTER SUBSTANCES

The technique of paper chromatography presented a quick method for the determination of the bitter principle composition of small quantities of material. Since these bitter principles are only slightly soluble in water, the method developed by Burton, Zaffaroni and Keutmann[12] for the chromatography of steroids on paper impregnated with formamide was used.

Good separations of bitter principles were obtained with a number of solvent systems. The spots were made visible by first spraying the papers with a solution of potassium permanganate dissolved in a saturated aqueous solution of copper acetate and then heating the chromatograms at 100° for two to three minutes. Permanent light blue spots appeared against a brown background. Amounts of 10-50$\gamma$ of bitter principle could be made visible on developed chromatograms (see Experimental for further details). In spite of all attempts to standardise the procedure and apparatus, we found that reproducibility of Rf values was poor (*cf.* also Burton, Zaffaroni and Keutmann[12], Schindler and Reichstein[13], Neher and Wettstein[14]). Factors such as the amount of formamide on the paper and the humidity of the air when drying the impregnated papers are responsible for much variation in the rate of movement of the spots. In all cases samples of reference substances were therefore run on the same strip of paper as unknown samples. Since most of the chromatograms

Fig. 1.



Cross between wild and cultivated water-melon: left, wild parent; right, Hawkesbury parent; centre, $F_1$ hybrid.

were developed for only one to two hours, temperature control was not applied. In one experiment (with ethylene dichloride as mobile phase) the average Rf values at 26° were higher than those found at 4° (see Table I).

TABLE I

| Solvent | Rf values of cucurbitacins | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | Dihydro-cucurbitacin A* | Elaterin† |
| Chloroform .. .. .. | 0·60 | 0·86 | 0·67 | 0·60 | 0·62 | 0·95 |
| Ethylene dichloride 4° .. | 0·27 | 0·82 | 0·20 | 0·24 | 0·43, 0·33 | 0·88 |
| Ethylene dichloride 26° .. | 0·45 | 0·86 | 0·40 | 0·43 | 0·64, 0·55 | 0·89 |
| Trichloroethylene .. | 0·04 | 0·70 | streaks | 0·04 | — — | streaks |
| Trichloroethane .. .. | 0·00 | 0·55 | 0·00 | 0·00 | 0·00, 0·10 | — |
| Chloroform-benzene 3:1 .. | 0·39 | 0·87 | 0·44 | 0·39 | 0·59, 0·47 | 0·89 |
| Chloroform-benzene 1:1 .. | 0·16 | 0·80 | streaks | 0·16 | 0·33, 0·19 | 0·83 |
| Chloroform-benzene 1:4 .. | 0·06 | 0·72 | — | — | — — | streaks |
| EtOAc-benzene 3:1 .. .. | 0·45 | 0·79 | — | 0·56 | 0·74, 0·60 | streaks |
| EtOAc-benzene 3:2 .. .. | 0·36 | 0·77 | — | 0·48 | — — | — |
| Benzene .. .. .. .. | 0·02 | 0·55 | 0·02 | 0·03 | — — | streaks |
| Chlorobenzene .. .. .. | 0·06 | 0·74 | streaks | 0·06 | — — | streaks |
| Toluene .. .. .. .. | 0·00 | 0·40 | — | — | — — | streaks |

* See Part I of this Series.
† Commercial sample (Merck and Co.), m.p. 228°.

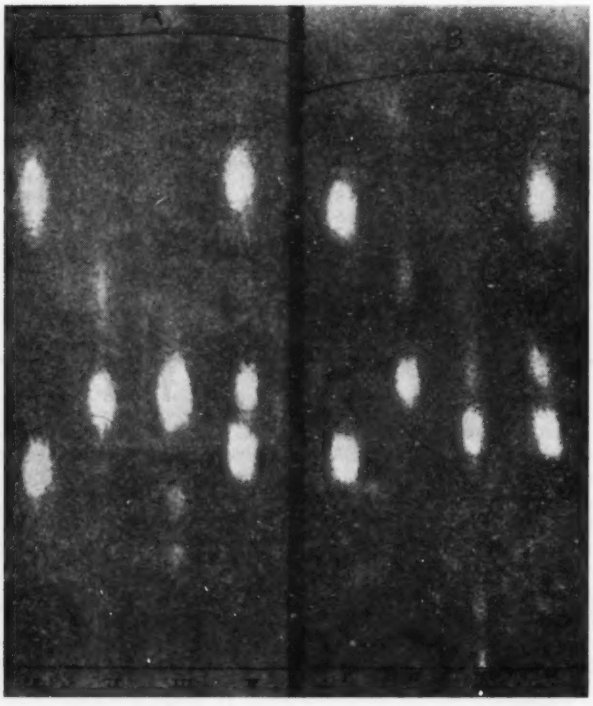
In the Table are presented average Rf values. They have no absolute significance, but give some indication of the relative positions of the spots on the paper. The Rf value for cucurbitacin A at 26° with ethylene dichloride is given as 0·45. This is the average of duplicate spots run on 12 separate chromatograms. The actual values varied from 0·39 to 0·54. The average Rf value of cucurbitacin B (0·86) for the same solvent was calculated from 12 chromatograms on which the Rf values varied from 0·76 to 0·91.

It is clear from Table I that all the bitter principles could be separated from one another by the selection of a suitable solvent system. Mixtures of ethyl acetate and benzene had good resolving power and were the only solvent systems capable of separating cucurbitacin A and D. With chloroform, both cucurbitacin D (m.p. 150°) and dihydro-cucurbitacin A (recrystallised twice) moved as single spots. In chloroform-benzene, ethyl acetate-benzene and ethylene dichloride both were resolved into two spots indicating an impurity in the cucurbitacin D preparation (see Fig. 2) and the fact that dihydrocucurbitacin A is a mixture (possibly of epimers).

The value of this technique can be illustrated by the following examples: samples of Rimington's[15] "cucumin", "leptodermin" and the "amorphous bitter principle" from a South West African *Cucumis* species (obtained through the courtesy of Professor D. G. Steyn, University of Pretoria) were spotted on to paper and developed with ethyl acetate-benzene (3:2). Both leptodermin and cucumin (see Fig. 2B) consisted mainly of cucurbitacin A together with other impurities which gave spots at lower Rf values. Cucumin may contain a small quantity of cucurbitacin D. Both samples were free from cucurbitacin B. The bitter principle from the South West African species probably consisted mainly of cucurbitacin D. Two other weaker spots appeared at lower Rf values (see Fig. 2A).

The value of this technique was also demonstrated when Professor D. G. Steyn of the University of Pretoria sent us *ca.* 2 ml. of a liquid which formed part of a potion which a Native medicine man gave to one of his patients with fatal results. From this liquid *ca.* 1 mg. of an amorphous white powder was isolated. When run on paper with chloroform, the presence of cucurbitacin A or D and of small quantities

Fig. 2.




Paper chromatograms of bitter substances. Solvent: ethyl acetate, 3; benzene, 2.

A. I. 100$\gamma$ cucurbitacin A + 100$\gamma$ cucurbitacin B.
II. 200$\gamma$ cucurbitacin D.
III. 200$\gamma$ amorphous bitter principle from S.W. African *Cucumis* species (ex Prof. C. Rimington).
IV. 100$\gamma$ cucurbitacin A + 100$\gamma$ cucurbitacin B + 100$\gamma$ cucurbitacin D.

B. I. 100$\gamma$ cucurbitacin A + 100$\gamma$ cucurbitacin B.
II. 200$\gamma$ cucurbitacin D.
III. 200$\gamma$ "cucumin" (ex Prof. C. Rimington).
IV. 100$\gamma$ cucurbitacin A + 100$\gamma$ cucurbitacin B + 100$\gamma$ cucurbitacin D.

of cucurbitacin B was indicated. This bitter-tasting potion was therefore probably prepared from *Cucumis myriocarpus* fruits.

Enough total bitter principle could be extracted (method described in Part I of this series) from a single *Cucumis* fruit weighing *ca.* 10 g. for a determination of its bitter principle composition by paper chromatography. In one experiment, 10λ of the fresh sap from a mature *Cucumis myriocarpus* fruit was spotted on paper and developed with ethyl acetate-benzene (3:1) mixture. A clear spot indicated the presence of cucurbitacin A. A survey of bitter wild and bitter cultivated fruits of the family Cucurbitaceae is at present being made in co-operation with the Division of Botany in Pretoria.

Only amorphous bitter principles have so far been isolated from the wild or the cultivated bitter water-melon. When spotted on paper, these hardly moved from the starting line, showing that they are quite different from any of the crystalline cucurbitacins. Cucurbitacin C has been isolated from a bitter line of *Cucumis sativus*, known as wild Hanzil medicinal cucumber in India, (planted from seeds presented by Dr. W. S. Barham, University of North Carolina, Raleigh, U.S.A.). So far we have not yet been able to obtain enough material of bitter cultivated cucumbers to identify the bitter principles present in these varieties.

It is possible to use the size of the spots on the chromatograms for the rough quantitative determination of the cucurbitacins. The quantitative estimation of the cucurbitacin content of plant material should be the basis for the investigation of a number of important biological problems.

## EXPERIMENTAL

Whatman No. 540 filter paper was preferred to Whatman No. 1, since, when impregnated, it was more resistant to tearing. Strips of filter paper were cut 10 cm. wide and 60 cm. long. A horizontal pencil line, representing the starting line, was drawn 6 cm. from the one end. Commercial formamide was purified by distillation through a short column under water pump vacuum. All other solvents were purified by the conventional methods.

The amount of formamide absorbed by the filter paper is critical. It is also very important that the stationary phase should be evenly distributed throughout the paper. Originally the papers were immersed in a 25% solution of a formamide in alcohol and hung up to dry. To prevent uneven distribution of the stationary phase by drainage of the solution to the lower end of the paper, the bottom 10 cm. was not impregnated. This dry strip of paper removed any excess of solution draining downwards. After drying in air for one hour, the papers were cut to the desired length (usually 30-45 cm.) and spotted (spots 2 cm. apart) with 5-10λ of a 2% solution of the bitter substance in chloroform (or alcohol), care being taken to keep the size of the spot as small as possible.

The arrangement of the apparatus was similar to that of Burton, Zaffaroni and Keutmann[12], who used the descending method of development. The atmosphere within the chamber was kept saturated with mobile phase by sheets of filter paper wetted with the solvent saturated with formamide. Before introducing the solvent, the papers were kept in the chamber for one hour. Since the mobile phase is very quickly saturated with the formamide absorbed on the paper, it was generally found unnecessary to saturate the solvents with the stationary phase before introducing them through a hole in the lid of the chamber. The solvents took from 1 to 2 hours to reach the bottom of the paper.

The developed chromatograms were dried in a current of air for 5 minutes and then evenly sprayed with a 0·5% solution of $KMnO_4$ in a saturated aqueous solution of copper acetate. Yellow spots appeared instantaneously against a red background. This reaction probably involves oxidation of the double bond, since it is not given by dihydrocucurbitacin A. Almost immediately, however, the whole paper turns brown and the spots are no longer visible. When these chromatograms

are heated for 2 to 3 minutes at 100°, blue spots appear against a brown-green background. These spots are permanent, but disappear on prolonged heating. The presence of formamide is necessary for the production of these spots. The sensitivity of the colour reaction differs for the various bitter substances. Ten $\gamma$ cucurbitacin A and 10$\gamma$ cucurbitacin B, after separation on paper, can still be detected with this reaction. Dihydrocucurbitacin A also produces a blue spot which is, however, weaker than that obtained from the same quantity of cucurbitacin A. Cucurbitacin C produces a strong initial yellow spot after spraying, but gives a weaker reaction after heating.

We also studied the production of fluorescent spots, visible in ultra-violet light after heating the papers with trichloroacetic acid, antimony trichloride or 15% phosphoric acid. Only weak spots were produced.

The authors are indebted to Mr. F. S. M. Gilham, now at the Cotton Experimental Station, Gatooma, Southern Rhodesia, for help with the breeding work, Mr. L. Novellie for helpful discussions in connection with paper chromatographic techniques, and Dr. D. A. Sutton for his interest in this investigation.

This paper is published with the permission of the S.A. Council for Scientific and Industrial Research, and the Chief, Division of Horticulture, Pretoria.

National Chemical Research Laboratory,
S.A. Council for Scientific and Industrial Research, Pretoria.

Horticultural Research Station, Pretoria,
Division of Horticulture, Department of Agriculture, Pretoria.

*Received June* 24, 1954.

## REFERENCES

[1] Steyn, D. G., *J. S. Afr. vet. med. Ass.*, 1932, **3**, 44.
[2] Steyn, D. G., *Onderstepoort J. vet. Sci.*, 1935, **4**, 399.
[3] Steyn, D. G., *J. R. sanit. Inst.*, 1936, **56**, 768.
[4] Steyn, D. G., *S. Afr. med. J.*, 1950, **24**, 713.
[5] Enslin, P. R., *J. Sci. Fd. Agric.*, 1954, **5**, 410.
[6] Pathak, G. and Singh, B., *Indian J. Genet.*, 1950, **10**, 28.
[7] Barham, W. S., *Proc. Amer. Soc. Hort. Sci.*, 1953, **62**, 441.
[8] Vogel, F., *Obst- u. Gemüseb.*, 1934, **80**, 49.
[9] Vogel, F., *NachrBl. dtsch. PflSch. Dienst*, 1934, **14**, 60, 66.
[10] Weiling, F., *Ber. dtsch. bot. Ges.*, 1953, **66**, 368.
[11] Whitacker, Th. W. and Bohn, G. W., *Econ. Bot.*, 1950, **4**, 52.
[12] Burton, R. B., Zaffaroni, A. and Keutmann, E. H., *J. biol. Chem.*, 1951, **188**, 763.
[13] Schindler, O. and Reichstein, T., *Helv. chim. acta.*, 1951, **34**, 108.
[14] Neher, R. and Wettstein, A., *Helv. chim. acta.*, 1952, **35**, 276.
[15] Rimington, C., *Onderstepoort J. vet. Sci.*, 1935, **4**, 65.

## OFFICE BEARERS AND COUNCIL—1954-1955—AMPSDRAERS EN RAAD

*President:*

S. S. Israelstam, M.Sc., Ph.D., F.R.I.C.;

*Immediate Past President/Vorige-president:*

Prof. L. D. C. Bok, M.Sc., Dr. rer Nat.

*Vice President/Vise-president:*

F. W. Hayes, F. R. I. C., A.M.I.Chem.E.;

*Hon. Secretary and Treasurer/Ere-sekretaris en Tesourier:*

C. F. Lindemann, B.Sc.

*Hon. Editor and Librarian/Ere-redakteur en Bibliotekaris:*

S. S. Israelstam, M.Sc., Ph.D., F.R.I.C.

*Members of Council/Raadslede:*
*S. Transvaal Section/Seksie:*

F. L. Clark, M.Sc.;
G. A. Sutton Clark, B.Sc.;
F. Hawke, M.Sc. (Eng.), Ph.D., F.R.I.C., A.M.I.Chem.E.;
H. A. E. Mackenzie, M.Sc., Ph.D., D.I.C., A.R.I.C.;
M. Mrost, M.Sc.

*Natal Section/Seksie:*

G. H. Butler, F.R.I.C., A. M. Inst. S.P.;
H. N. C. MacLenan, B.Sc.;
A. F. Strangman, M.Sc.

*Pretoria Section/Seksie:*

G. W. Perold, D.Sc., D.Sc.Tech.;
C. C. van der Merwe, M.Sc.;
J. van Garderen, D.Sc., F.R.I.C.

*E. Province Section/O. Provinsie Seksie:*

F. G. Thompson, B.Sc.;
R. Schauder, B.Sc.

*W. Province Section/W. Provinsie Seksie:*

E. N. Costa, B.Sc.;
P. H. de V. de Villiers, M.Sc.;
Prof. E. F. C. H. Rohwer, M.Sc., Dr. rer. Nat.;

## VIR DIE AANDAG VAN OUTEURS

Die Joernaal van die Suid-Afrikaanse Chemiese Instituut word gewy aan die publikasie van oorspronklike navorsing in alle vertakkings van chemie en chemiese tegnologie.

Alle bydraes moet in triplikaat ingehandig word; indien daar grafieke is moet een stel noukeurig met *Oosindiese ink afgeteken word op gladde wit Bristol-blad of tekenpapier, of op afrekdoek* —die ander twee stelle kan afdrukke wees, of met potlood geteken word.

*Artikels moet so saaklik moontlik wees, verenigbaar met duidelikheid*, en moet nie onnodige eksperimentele besonderhede bevat nie. Artikels moet *in tikskrif wees—dubbel spasiëring*—net aan een kant van die papier, met 'n behoorlike kantruimte aan die linkerkant. Die papier moet nie-absorberend wees.

Die buitemate van plate—insluitende die ruimte vir byskrif—moet hoogstens 14 duim by 10 duim wees, en liefs nie minder as 7 duim by 5 duim nie. Die verhouding 1·4:1·0 moet in elk geval nagekom word. Byskrif en letters—hetsy in die kantruimte of in die tekening self—moet nie met ink gedoen word nie, *maar liggies met gewone potlood of uitveesbare bloupotlood*.

Elke artikel moet voorafgegaan word deur 'n opsomming in elk van die twee offisiële landstale, en die opsomming wat tweede verskyn moet in dieselfde taal as die artikel wees. Die opsomming moet in eenvoudige taal 'n kort en saaklike uiteensetting gee van die oogmerke van die ondersoek, die resultate wat behaal is, en die gevolgtrekkings. Die opsomming moet ongeveer 50-250 woorde beslaan en mag in elk geval nie meer as 5 persent van die artikel wees nie.

Verwysings moet in numeriese opeenvolging aan die end verskyn, in die volgorde: skrywer se van; skrywer se voorletters; joernaal; datum; deel; bladsy; die ooreenstemmende verwysingsnommers moet in volgorde in die teks verskyn as kopskrifte.

Verwysings na boeke moet as volg aangedui word: skrywer se van; skrywer se voorletters; titel; uitgawe; uitgewer; datum van publikasie; bladsy.

Simbole en afkortings moet volgens B.S. 813: 1938 wees.

Bydraers moet afskrifte hou van al hulle mededelings.

*Outeurs word versoek om hulle bydraes so op te stel dat dit ooreenstem met die gewone trant konvensie en kernagtigheid van hierdie joernaal.*

Dui asseblief aan waarheen die drukproewe moet gestuur word.

Outeurs kan verdere voorskrifte en aanwysings, i.v.m. die opstel van 'n artikel, aanvra.

Rig asseblief alle korrespondensie aan die Redakteur van die Joernaal, Suid-Afrikaanse Chemiese Instituut, Kelvinhuis, h/v Marshall- en Hollardstraat, Johannesburg.

## NOTICE TO AUTHORS

The Journal of the South African Chemical Institute is designed for the publication of original work in all branches of chemistry and chemical technology.

All papers must be submitted in triplicate: where diagrams are included one set of these must be carefully drawn, *in Indian ink, on smooth white Bristol board or paper, or on tracing cloth*, the remaining two copies may be pencil drawings or prints.

*Papers should be as brief as possible compatible with clarity*, and no unnecessary experimental details should be included. All papers must be *typewritten in double line spacing*, on one side only of the paper, with a good margin on the left hand side. The paper should be non-absorbent.

The total external dimensions of illustrations (including space to be taken up by lettering) should not exceed 14 inches by 10 inches and preferably should not be less than 7 inches by 5 inches—the proportion 1·4:1·0 being in all cases maintained. Lettering on drawings, whether on the margin or in the body of the drawing must not be in ink but must be inserted *lightly, in blue pencil or erasable lead pencil*.

Every paper must be preceded by two summaries, one in each official language; the second summary being in the same language as that of the paper. Each summary should set forth concisely and in simple language the objects and purpose of the investigation, the results obtained and conclusions drawn. These should normally be from 50 to 250 words in length and, in any case, should not exceed 5 per cent. of the paper.

References should appear at the end of the paper, in numerical order in the following sequence: author's surname; author's initials; journal; date; volume number; page; the necessary reference numbers should appear, in sequence, as superscripts in the text.

Where reference is made to books the following sequence should be adhered to: author's surname; author's initials; title; edition; publisher; date of publication; page.

Symbols and abbreviations used should conform with B.S. 813: 1938.

Authors should retain copies of their communications.

*Authors are requested to arrange their papers to conform, as far as possible, with the normal style, conventions and conciseness of this journal.*

The address to which proofs are to be sent should be written on every paper.

Directions to authors regarding the preparation of papers will be forwarded on application.

All communications should be addressed to the Editor of the Journal The South African Chemical Institute, Kelvin House, cor. Marshal and Hollard Streets, Johannesburg.

C.P.W.-O.M.S. 8